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NON-A, NON-B HEPATITIS VIRUS GENOME, POLYNUCLEOTIDES, POLYPEPTIDES, ANTIGEN, ANTIBODY AND DETECTION SYSTEMS. ;

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ABSTRACT:

Non-A, non-B hepatitis (NANB hepatitis) virus RNA and its corresponding polypeptide, related antigen, antibody, and detection systems for detecting NANB hepatitis antigen or antibodies.

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(54) Non-A, Non-B Hepatitis virus genome, polynucleotides, polypeptides, antigen, antibody and detection systems.

(57) Non-A, non-B hepatitis (NANB hepatitis) virus RNA and its corresponding polypeptide, related antigen, antibody, and detection systems for detecting NANB hepatitis antigen or antibodies.

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R ference To A Related Application

Th present application is a continuation-in-part of our cop nding U.S. Pat nt Application Serial No. 07/866,045, filed on April 9, 1992, which is incorporated by reference in its entirety.

Background of the Invention

The present invention concerns non-A, non-B hepatitis (hereinafter called NANB hepatitis) virus genome, polynucleotides, polypeptides, related antigen, antibody and detection systems for detecting NANB antigens or antibodies.

Viral hepatitis of which DNA and RNA of the causative viruses have been elucidated, and their diagnosis and even prevention in some have been established, are hepatitis A and hepatitis B. The general name NANB hepatitis was given to the other forms of viral hepatitis.

Post-transfusion hepatitis was remarkably reduced after introduction of diagnostic systems for screening hepatitis B in transfusion bloods. However, there are still an estimated 280,000 annual cases of post-transfusion hepatitis caused by NANB hepatitis in Japan.

NANB hepatitis viruses were recently named C,D and E according to their types, and scientists started a world wide effort to conduct research for the causative viruses and subsequent extermination of the causative viruses.

In 1988, Chiron Corp. claimed that they had succeeded in cloning RNA virus genome, which they termed hepatitis C virus (hereinafter called HCV), as the causative agent of NANB hepatitis and reported on its nucleotide sequence (British Patent 2,212,511 which is the equivalent of European Patent Application 0,318,216). HCV (C100-3) antibody detection systems based on the sequence are now being introduced for screening of transfusion bloods and for diagnosis of patients in Japan and in many other countries. The detection systems for the C100-3 antibody have proven their partial association with NANB hepatitis; however, they capture only about 70% of carriers and chronic hepatitis patients, or they fail to detect the antibody in acute phase infection, thus leaving problems yet to be solved even after development of the C100-3 antibody by Chiron Corp.

The course of NANB hepatitis is troublesome and most patients are considered to become carriers, then to develop chronic hepatitis. In addition, most patients with chronic hepatitis develop liver cirrhosis, then hepatocellular carcinoma. It is therefore very imperative to isolate the virus itself and to develop effective diagnostic reagents enabling earlier diagnosis.

The presence of a number of NANB hepatitis which cannot be diagnosed by Chiron's C100-3 antibody detection kits suggests a possibility of a difference in subtype between Chiron's HCV and Japanese NANB hepatitis virus.

In order to develop NANB hepatitis diagnostic kits of more specificity and to develop effective vaccines, it becomes an absolutely important task to analyze each subtype of NANB hepatitis causative virus at its genetic and corresponding amino acid level.

Summary of the Invention

An object of the present invention is to provide the nucleotide sequence coding for the structural protein of NANB hepatitis virus and, with such information, to analyze amino acids of the protein to locate and provide polypeptides useful as antigen for establishment of detection systems for NANB virus, its related antigens and antibodies.

A further object of the present invention is to locate polynucleotides essential to treatment, prevention and diagnosis, and polypeptides effective as antigens, by isolating NANB hepatitis virus RNA from human and chimpanzee virus carriers, cloning the cDNA covering the whole structural gene of the virus to determine its nucleotide sequence, and studying the amino acid sequence of the cDNA. As a result, the inventors have determined the nucleotides of the whole genome of a strain of NANB virus called HC-J6 and a strain called HC-J8. NANB hepatitis virus genome of HC-J6 and HC-J8 differ from that of Chiron's HCV.

Brief Description of the Drawings

Figure 1 shows the restriction map and structure of the coding region of NANB hepatitis virus genome (HC-J6) and positions of clones. C, E, NS-1, NS-2, NS-3, NS-4 and NS-5 are th abbreviation of core, nvelope, non-structure-1, -2, -3, -4 and -5.

Figures 2 to 4 show method of determination of the nucleotide sequence of 5' t rminus of NANB h patitis virus genom of strains HC-J1, HC-J4 and HC-J6 respectively.

Figur 5 shows the m thod of d t rmination of th nucleotid sequence of 3' terminus of HC-J6 genome. Solid lines show nucleotide sequences determined by clones from libraries of bacteriophage lambda gt10, and broken lines show nucleotide sequences determined by clones obtained by PCR.

Figure 6 shows the structure of coding region of NANB hepatitis virus genome (HC-J8) and positions of clones. Regions a to n indicate positions of amplification by PCR.

Detailed Description of the Invention

The present invention provides NANB hepatitis virus genome RNA for strain HC-J6 (sequence list 1) consisting of 340 nucleotides on the 5' terminus that follow an open reading frame consisting of 9099 nucleotides coding for the structural protein and non-structural protein that follow a noncoding region consisting of 150 nucleotides containing an U-stretch consisting of 108 uracils on the 3' terminus of NANB hepatitis virus, and NANB hepatitis virus genome having substantially the nucleotide sequence of sequence list 1.

The present invention provides polynucleotide N-9589 (strain HC-J6) comprising the DNA nucleotide sequence of sequence list 2; cDNA clone J6-ø81 comprising the nucleotide sequence of sequence list 3; cDNA clone J6-ø8 comprising the nucleotide sequence of sequence list 4; and NANB hepatitis virus polynucleotides having substantially the sequence of nucleotides of NANB hepatitis virus nucleotides shown in sequence lists 2 through 4.

The invention provides polypeptide coded for by genome or polynucleotide of HC-J6 above, polypeptide P-J6-3033, comprising the polypeptide sequence of sequence list 5, polypeptides produced by using recombinant genome, recombinant polynucleotides and recombinant cDNA of whole or a part of cDNA above, and polyclonal or monoclonal antibodies against the polypeptides described above.

The present invention also provides NANB hepatitis virus genome for strain HC-J8 comprising sequence list 6, NANB hepatitis virus RNA consisting of noncoding region consisting of 341 nucleotides on 5' terminus followed by an open reading frame consisting of 9099 nucleotides coding for the structural protein and non-structural protein followed by a noncoding region consisting of 71 nucleotides containing an U-stretch consisting of 30 uracils on 3' terminus of NANB hepatitis virus comprising sequence list 6, and NANB hepatitis virus genome having substantially the nucleotide sequence of sequence list 6.

The present invention provides polynucleotide N-9511 for strain HC-J8 comprising the DNA nucleotide sequence of sequence list 7 and NANB hepatitis virus polynucleotide having substantially the sequence of nucleotides of NANB hepatitis virus nucleotides comprising sequence list 7.

The invention provides polypeptide coded for by genome or polynucleotide of HC-J8 above, polypeptide P-J8-3033, comprising the polypeptide sequence of sequence list 8 and polypeptide P-J8-3033-2 comprising the polypeptide sequence of sequence list 9, polypeptides produced by using recombinant genome, recombinant polynucleotides and recombinant cDNA of whole or a part of cDNA above, and polyclonal or monoclonal antibodies against the polypeptides described above.

The present invention, furthermore, provides NANB hepatitis diagnostic system using polypeptides or antibodies described above.

In the method described below, NANB hepatitis virus RNA of the present invention was obtained and its nucleotide sequence was determined.

Plasma samples (HC-J1, HC-J4, HC-J6 and HC-J8) were obtained from human and chimpanzee. HC-J1, HC-J6 and HC-J8 were obtained from Japanese blood donors who had tested positive for HCV antibody. HC-J4 was obtained from the chimpanzee subjected to the challenge test but was negative for Chiron's C100-3 antibody previously mentioned.

RNA was isolated from each of the plasma samples. Following the study of 5' terminus of approximately 2,500 nucleotides and 3' terminus of approximately 1,100 nucleotides disclosed in Japanese patent application No. 196175/91, the inventors have completed the study of the region coding for non-structural protein of strain HC-J6 and the study of the full length sequence of 9,589 nucleotides of HC-J6 genome RNA and have completed the study of th region coding for non-structural protein of strain HC-J8 and the study of the full length sequence of 9,589 nucleotides of HC-J8 genome RNA.

As described in the Example below, strain HC-J6 had a 5' noncoding region consisting of 340 nucleotides, and strain HC-J8 had a 5' noncoding region consisting of 341 nucleotides, followed by region coding for structural protein and region coding for non-structural protein.

Concerning the 3' terminus, strain HC-J6 was found to have a region consisting of 150 nucleotides containing an U-stretch consisting of 108 uracils following after the region coding for non-structural protein

and strain HC-J8 was found to hav a region consisting of 71 nucleotides containing an U-stretch consisting of 30 uracils following after th region coding for non-structural protein.

Th coding region starting with ad nine (341st nucl otid from the 5' terminus for strain HC-J6 and 342nd nucleotide from the 5' terminus for strain HC-J8) was found to have a long *Open Reading Frame* consisting of 9099 nucleotides which codes for 3033 amino acids. HCV or hepatitis C virus is supposed to be closely allied to flavivirus in regard to its genetic structure. The coding of the NANB hepatitis virus genome of the present invention was considered to be consisting of regions named C (core), E (envelope), NS-1 (non-structural-1), NS-2 (non-structural-2), NS-3 (non-structural-3), NS-4 (non-structural-4) and NS-5 (non-structural-5).

As compared with the sequence of HCV disclosed in the European Patent Application by Chiron Corp. (Publication No. 388,232), homology of sequences of the strain HC-J6 was 67.9% for the full nucleotide sequence and 72.3% for the full amino acid sequence, and homology of sequences of the strain HC-J8 was 66.4% for the full nucleotide sequence and 71.0% for the full amino acid sequence.

From an examination of homology for regions, the homology of nucleotide sequences (strain HC-J6) of the 5' terminal noncoding region was 94.4% and that of the amino acid sequences of the C region was 90.1%, showing comparatively high homology; on the other hand, concerning lower stream than envelope, homologies of amino acid sequence were found to be as low as 60.4% for E, 71.1% for NS-1, 57.8% for NS-2, 81.1% for NS-3, 73.1% for NS-4, and 69.9% for NS-5. As a result, HC-J6 strain was found to be significantly different from HCV strain found by Chiron Corp.

From an examination of homology for regions, the homology of nucleotide sequences (strain HC-J8) of the 5' terminal noncoding region was 93.8% and that of the amino acid sequences of the C region was 90.1%, showing comparatively high homology; on the other hand, concerning lower stream than envelope, homologies of amino acid sequence were found to be as low as 54.7% for E, 73.1% for NS-1, 55.6% for NS-2, 81.3% for NS-3, 72.1% for NS-4, 67.3% for NS-5, and 25.9% for 3' terminal noncoding region. As a result, HC-J8 strain was found to be significantly different from HCV strain found by Chiron Corp.

From the comparison of amino acid sequence of HC-J6 strain with strain HC-J1 (American type) and strain HC-J4 (Japanese type) disclosed by the inventors (Japan. J. Exp. Med. (1990), 60: 167-177), homology in the core region was more than 90% for each strain while that in the envelope region was 60.9% for HC-J1 and 53.1% for HC-J4. Thus, in the present invention, strain HC-J6 was found to be a different type of virus than strains HC-J1 or HC-J4.

From the comparison of amino acid sequence of HC-J8 strain with strain HC-J1 (type I) and strain HC-J4 (type II), homology of approximately 3,000 nucleotides of 5' terminus was 70.1% for HC-J1 and 67.1% for HC-J4, and from the comparison of all nucleotides with HC-J6 (type III) genome homology was as low as 76.9%. On the other hand, HC-J8 showed high homology with strain HC-J7 (type IV) disclosed in Japanese patent application 196175/91 as 93.1% for approximately 3,000 nucleotides of 5' terminus.

Nucleotides among stains assumed to belong to same type were supposed to show high homology. For example, homology of 95.6% for approximately 3,000 nucleotides of 5' terminus between HCV disclosed by Chiron Corp. and HC-J1 appears to show that they should be classified into type I. On the other hand, low homology of HC-J8 with HCV, HC-J1, HC-J4 and HC-J6 appeared to show that it was not to be classified into type I, II or III, but into type IV (the same as HC-J7).

Strain HC-J8 has some mutations in the nucleotides as shown in sequence lists 6 and 7 by symbols M, R, W, S, Y, K and B. It also can be easily understood that it has some mutations of amino acids from comparison of sequences in sequences lists 8 and 9. Mutation of nucleotides was observed up to approximately 1.4% in the whole genome and that of amino acids was observed up to approximately 1.7% in whole ORF. Thus the present invention includes genomes, polynucleotides and polypeptides of strain HC-J8 having some mutations.

In addition, envelope (E) region (576 nucleotides/192 amino acids of amino acids 192-383) and NS-1 region (1050 nucleotides/350 amino acids of amino acids 384-733) having many mutations in HC-J8 are called hyper-variable region since mutations were observed as 20 nucleotides/7 amino acids (3.47%/3.64%) in E region and 37 nucleotides/19 amino acids (3.52%/5.42%) in NS-1 region. According to these findings, the present invention can be recognized to include genomes and polypeptides coded for by the genomes of strain HC-J8 having mutations of 3.5% to 5.5% in those regions.

The genome, polynucleotide, and cDNA clones of the present invention can be used as material to produce peptides of the invention by integration into a host genome, e.g. *E. coli* or *Bacillus*, by means of known genetic engineering techniques.

Polypeptides of the inv ntion are useful as material for diagnostic agents to detect NANB hepatitis antibodies with high specificity and as material to produce polyclonal and monoclonal antibodies by known techniques.

Polyclonal and monoclonal antibodies of the invention are useful as materials for diagnostic agents to detect NANB hepatitis antigens with high specificity.

A detection system using each polypeptide of the present invention or polypeptide with partial replacement of amino acids, and a detection system using monoclonal or polyclonal antibodies to such polypeptides, are useful as diagnostic agents of NANB hepatitis with high specificity and are effective to screen out NANB hepatitis virus from transfusion bloods or blood derivatives. The polypeptides, or antibodies to such polypeptides, can be used as a material for a vaccine against NANB hepatitis virus.

It is well known in the art that one or more nucleotides in a DNA sequence can be replaced by other nucleotides in order to produce the same protein. The present invention also concerns such nucleotide substitutions which yield DNA sequences which code for polypeptides as described above. It is also well known in the art that one or more amino acids in an amino acid sequence can be replaced by equivalent other amino acids, as demonstrated by U.S. Patent No. 4,737,487 which is incorporated by reference, in order to produce an analog of the amino acid sequence. Any analogs of the polypeptides of the present invention involving amino acid deletions, amino acid replacements, such as replacements by other amino acids, or by isosteres (modified amino acids that bear close structural and spatial similarity to protein amino acids), amino acid additions, or isosteres additions can be utilized, so long as the sequences elicit antibodies recognizing NANB antigens.

Examples of application of this invention are shown below, however, the invention shall in no way be limited to those examples.

Examples

The 5' terminal nucleotide sequence and amino acid sequence of NANB hepatitis virus genome were determined in the following way:

(1) Isolation of RNA

RNA of the sample (HC-J1, HC-J6, HC-J8) from plasma of Japanese blood donor testing positive for HCV (C100-3) antibody (by Ortho HCV Ab ELISA, Ortho Diagnostic System, Tokyo), and that of the sample (HC-J4) from the chimpanzee challenged with NANB hepatitis for infectivity and negative for HCV antibody were isolated in the following method:

Each plasma sample was added with Tris chloride buffer (10 mM, pH 8.0) and centrifuged at 68 x 10^3 rpm for 1 hour. Its precipitate was suspended in Tris chloride buffer (50 mM, pH 8.0) containing 200 mM NaCl, 10 mM EDTA, 2% (w/v) sodium dodecyl sulfate (SDS), and proteinase K 1 mg/ml, incubated at 60 °C for 1 hour, then their nucleic acids were extracted by phenol/chloroform and precipitated by ethanol to obtain RNA.

(2) HC-J1 and HC-J8 cDNA Synthesis

After heating the RNA isolated from HC-J1 or HC-J8 plasma at 70 °C for 1 minute, this was used as a template; 10 units of reverse transcriptase (cDNA Synthesis System Plus, Amersham Japan) and 20 pmol of oligonucleotide primer (20 mer) were added and incubated at 42 °C for 1.5 hours to obtain cDNA. Primer #8 (5'- GATGCTTGCGGAAGCAATCA - 3') was prepared by referring to the basic sequence shown in European Patent Application No. 88310922.5, which is relied on and incorporated herein by reference.

(3) cDNA Was Amplified by the following Polymerase Chain Reaction (PCR)

cDNA was amplified for 35 cycles according to Saiki's method (Science (1988) 239: 487-491) using Gene Amp DNA Amplifier Reagent (Perkin-Elmer.Cetus) on a DNA Thermal Cycler (Perkin-Elmer.Cetus).

For cDNA synthesis and for PCR for HC-J8, synthesized primers disclosed in Japanese patent application 153402/90 and those based on HC-J1, HC-J4 and HC-J6 genomes disclosed in Japanese patent applications 196175/91 and below were utilized.

(4) Determination of 5' Terminal Nucleotide Sequence of HC-J1 and HC-J4 by Assembling cDNA Clones

As shown in Figures 2 and 3, nucleotide sequences of 5' termini of the genomes of strains HC-J1 and HC-J4 were determined by combined analysis of clones obtained from the cDNA library constructed in bacteriophage λgt10 and clones obtained by amplification of HCV specific cDNA by PCR.

Figures 2 and 3 show 5' t rmini of NANB hepatitis virus g nom togeth r with cl avag site by restriction ndonuclease and sequenc of primers used. In the figur s, solid lines are nucl otid sequ nc s determin d by clon s from bacteriophag λgt10 library whil dotted lin s show sequ nc s determined by clones obtained by PCR.

A 1656 nucleotide sequence of HC-J1 spanning nt454-2109 was determined by clone ø41 which was obtained by inserting the cDNA synthesized with the primer #8 into λgt10 phage vector (Amersham).

Another primer #25 (5'- TCCCTGTTGCATAGTTCACG -3') corresponding to nt824-843 was synthesized based on the ø41 sequence, and four clones (ø60, ø61, ø66 and ø75) were obtained to cover the upstream sequence nt18-843.

(5) Determination of 5' Terminal Nucleotide Sequence of HC-J6.

The nucleotide sequence of the 5' terminus of strain HC-J6 was determined from analysis of clones obtained by PCR amplification as shown in Figure 4.

Isolation of RNA from HC-J6 and determination of its sequence was made in the same manner as described in (2) above. Sequences in the range of nt24-2551 of the RNA were determined from consensus sequence of respective clones obtained by amplification by PCR using each pair of primers based on nucleotide sequence of HC-J4.

nt24-826

#32 (5'-ACTCCACCATAGATCACTCC-3')

#122 (5'-AGGTTCCCTGTTGCATAATT-3')

Clones: C9397, C9388, C9764

nt732-1907

#50 (5'-GCCGACCTCATGGGGTACAT-3')

#128 (5'-TCGGTCGTGCCCACTACCAC-3')

Clones: C9316,C9752,C9753

nt1847-2571

#149 (5'-TCTGTGTGTGGCCCAGTGTA-3')

#146 (5'-AGTAGCATCATCCACAAGCA-3')

Clones: C11621,C11624,C11655

In order to determin further upstream of the 5' terminus, antisens primer #36 (5'- AACACTACTCGG-CTAGCAGT -3') corresponding to nt246-265, followed by dAs were added to 5' terminus of cDNA using terminal deoxynucleotidyl transf rase, and one-sided PCR amplification was mad twice as described below.

cDNA was amplified for 35 cycles as first stage PCR using oligo dT primer (20-mer) and antis ns primer #48 (5'-GTTGATCCAAGAAAGGACCC -3') of nt188-207, followed by th second stag of PCR by 30 cycle amplification using th first PCR product as a template, oligo dT primer (20 -mer) and antisense

primer #109 (21-mer; 5'-ACCGGATCCGCAGACCACTAT-3') corresponding to nt140 to 160. The obtained PCR product was subcloned to M13 phage vector.

Nucl otid sequ nce from nt1 to 23 was determined from cons nsus sequenc of 13 isolated clones C9577, C9579, C9581, C9587, C9590, C9591, C9595, C9606, C9609, C9615, C9616 and C9619 obtained above which were considered having complete 5' terminus.

(6) Determination of nucleotide sequence of HC-J6 middle region.

cDNA library was constructed with using λgt10 according to the method described in (2) above from 100ml of HC-J6 plasma as a starting materials. Primers #162 and #81 were prepared for synthesis by referring to the basic sequence shown in the European Patent Application Publication No. 318,216. Clones were selected by plaque hybridization.

Nucleotide sequence from 2552 to 8700 was determined from consensus sequence of four obtained cDNA clones ø2 (nt6996 to 8700), ø6(nt6485 to 8700), ø8(nt6008 to 8700) and ø81 (nt2199 to 6168) as shown in Figure 1. Clones ø81 and ø8 were found to have nucleotide sequences shown in sequence lists 3 and 4 respectively.

(7) Determination of 3' terminal nucleotide sequence of HC-J6 strain.

As shown in Figure 5, the nucleotide sequence of the 3' terminus of HC-J6 genome was determined by analysis of clones obtained by amplification of HCV specific cDNA by PCR.

Nucleotide sequence of HC-J6 from nt8701 to 9241 was determined from consensus sequence of three clones consisting of 938 nucleotides, C9760, C9234 and C9761, obtained by amplification of sample using primer #80 (5'-GACACCCGCTGTTTTGACTC-3') and #60 (5'-GTTCTTACTGCCCAGTTGAA-3').

Nucleotide sequence of 3' terminus down stream from nt9242 was determined in the method described below.

Isolation of RNA from HC-J6 was made in the same manner as described in (1) above. The obtained RNA was added poly (A) to its 3' terminus using poly (A) polymerase and cDNA was synthesized using oligo $(dT)_{20}$ as a primer, and obtained cDNA was provided to PCR as a template.

First PCR product was made with using #97 (5'-AGTCAGGGCGTCCCTCATCT-3') as a sense primer and oligo $(dT)_{20}$ as an antisense primer. Second PCR product was made with using #90 (5'-GCCGTTTGCGGCCGATATCT-3') corresponding to downstream sequence of #97 as a sense primer, and oligo $(dT)_{20}$ as an antisense primer as well as first PCR product. PCR product obtained by two step amplification was smoothened on both ends by treatment with T_4DNA polymerase, followed by phosphorylation of 5'terminus by T_4 polynucleotide kinase. The obtained product was subcloned into Hinc II position of M13mp19 phage vector.

Nucleotide sequence of 3' terminus was determined from consensus sequence of 19 obtained clones, C10311, C10313, C10314, C10320, C10322, C10323, C10326, C10328, C10330, C10333, C10334, C10336, C10337, C10345, C10346, C10347, C10349, C10350 and C10357.

As a result, the nucleotide sequence of cDNA to HC-J6 genome RNA was determined as shown in sequence list 2, and full sequence of genome RNA was determined as shown in sequence list 1.

(8) Determination of amino acid sequences.

According to the nucleotide sequence of the genome of strain HC-J6, determination was made of sequence of coded region starting with ATG. As a result, HC-J6 genome was found to have a long *Open Reading Frame* coding for polypeptide precursor consisting of 3033 amino acid residues.

(9) Determination of 5' terminal nucleotide sequence of HC-J8

As shown in Figure 6, the nucleotide sequence of 5' terminus of HC-J8 genome (a region) was determined by analysis of clones obtained by amplification of HCV specific cDNA by PCR.

Single-stranded cDNA was synthesized using antisense primer #36 (5'-AACACTACTCGGCTAGCAGT-3') of nt246 to 265 in the same manner as (2) above, then it was added with dATP tail at its 3' terminus by terminal deoxynucleotidyl transferase, then amplified by one-sided PCR in two stages.

That is, in th first stag , antisense prim r #48 (5'-GTTGATCCAAGAAAGGACCC-3') of nt188 to 207 was used with s nse primer selected from non-specific primer #165 (5'-AAGGATCCGTCGACATCGATAAT-ACG $(A)_{17}$-3') and #171 (5'-AAGGATCCGTCGACATCGATAATACG$(T)_{17}$-3') to amplify the dA-tailed cDNA

by PCR for 35 cycles; and in the second stage, using the product of the first-stage PCR as a template, non-specific primer #166 (5' AAGGATCCGTCGACATCGAT -3') and antisense primer #109 (21-mer; 5'-ACCG-GATCCGCAGACCACTAT -3') were added to initiate PCR for 30 cycles. The product of PCR was subcloned to M13 phage vector.

Thirteen independent clones (poly dT-tailed: C14951,C14952, C14953, C14958, C14960, C14968, C14971, C14972 and C14974; poly dA-tailed: C14987, C14996, C14999 and C15000) were obtained (each considered having complete length of 5' terminus), and the consensus sequence of nt1-139 of the respective clones was determined.

(10) cDNA amplification of ORF region and 3' terminus by PCR

As shown in Figure 6, the nucleotide sequence of downstream from nt140 of HC-J8 genome was determined by analysis of clones obtained by amplification of HCV specific cDNA by PCR.

Single-stranded cDNAs to HC-J8 RNA were synthesized in the same manner as (2) above using antisense primers described below, then they were amplified by PCR using sense and antisense primers described below. Each product of PCR was subcloned to M13 phage vector, then consensus sequence of the respective clones of each region was determined.

The primers for cDNA synthesis and PCR amplification, and the numbers of obtained clones are shown below for each region. Alphabetical symbol of each amplified region corresponds to that in Figure 6.

b region

nt45-847

Primer for cDNA synthesis: #122 (5'-AGGTTCCCTGTTGCATAATT-3')

Primer for PCR: sense: #32A (5'-CTGTGAGGAACTACTGTCTT-3')

antisense #122

Clones: C15221,C15222,C15223

c region

nt732-1354

Primmer for cDNA synthesis:#54 (5'-ATCGCGTACGCCAGGATCAT-3')

Primer for PCR: sense: #50 (5'-GCCGATCTCATGGGGTACAT-3')

antisense:#54

Clones: C15256,C15257,C15258

d region

nt1300-1879

Primer for cDNA synthesis: #199 (5'-GGGGTGAAACAATACACCGG-3')

Primer for PCR: sense: #205 (5'-GGGACATGATGATCAACTGG-3')

antisense: #199

Clones: C14221,C14222,C14223

e region

nt1833-2518

Primer for cDNA synthesis: #146 (5'-AGTAGCATCATCCACAAGCA-3')

Primer for PCR: sense: #150 (5'-ATCGTCTCGGCTAAGACGGT-3')

antisense: #146

Clones: C11535,C11540,C11566

f region

nt2433-3451

Primer for cDNA synthesis: #170 (5'-GCATAAGCAGTGATGGGGGC-3')

Primer for PCR: sense: #160 (5'-CAGAACATCGTGGACGTGCA-3')

antisense: #170

Clones: C15348,C15349,C15356

g region

nt3404-4300

Primer for cDNA synthesis: #225 (5'-TCGCATATGATGATGTCATA-3')

Primer for PCR: sense: #238 (5'-CTACACCTCCAAGGGGTGGA-3')

antisense: #225

Clones: C15701,C15702,C15703

h region

nt4221-5015

Primer for cDNA synthesis: #216 (5'-GTGGTCTAGACATACGGGCA-3')

Primer for PCR: sense: #230 (5'-CCCATCACGTACTCCACATA-3')

antisense: #216

Clones: C15391,C15392,C15393

i region

nt4695-5062

Primer for cDNA synthesis: #210 (5'-GCATCTATGTGTGTGAGGCC-3')

Primer for PCR: sense: #209 (5'-TTCGACTCCGTGATCGACTG-3')

antisense: #210

Clones: C14087,C14088,C14089

j region

nt5021-6169

Primer for cDNA synthesis: #162 (5'-TCCGACTCCGTCACGTAGTG-3')

Primer for PCR: sense: #227 (5'-GTTCTGGGAAGCGGTCTTTA-3')

antisense: #162

Clones: C15421,C15422,C15423

k region

nt6027-6889

Primer for cDNA synthesis: #232 (5'-GATGGGTCTGTTAGCATGGA-3')

Primer for PCR: sense: #242 (5'-TTGGTAGTGGGAGTCATCTG-3')

antisense: #232

Clones: C15733,C15734,C15735

l region

nt6834-7735

Primer for cDNA synthesis #239 (5'-ATCGGTAACTTCTCCTCTTC-3')

Primer for PCR: sense: #241 (5'-CCTTGCGATCCTGAACCTGA-3')

antisense:#239

Clones: C15798,C15799,C15800

m region

nt7656-8630

Primer for cDNA synthesis: #222 (5'-GACCAGGTCGTCTCCACACA-3')

Primer for PCR: sense: #229 (5'-GTCGTGTGCTGCTCCATGTC-3')

antisense: #222

Clones: C15376,C15378,C15381

n region

nt8325-9511

Primer for cDNA synthesis: #165

Primer for PCR: sense: #80 (5'-GACACCCGCTGTTTTGACTC-3')

non-specific: #165

Clones: C15270,C15271,C15272

From the analysis described above, full nucleotide sequence of cDNA to HC-J8 was determined as shown in sequence list 7, then full nucleotide sequence of HC-J8 genome RNA as shown in sequence list 6. Two amino acid sequences shown in sequence lists 8 and 9 represent those coded for by HC-J8 genome.

Utilizing known immunological techniques, it is possible to determine epitopes (e.g., from the core region, etc.) from the polypeptides of sequence lists 5, 8 and 9. Determination of such epitopes of the NANB hepatitis virus opens access to chemical synthesis of the peptide, manufacturing of the peptide by genetic engineering techniques, synthesis of the polynucleotides, manufacturing of the antibody, manufacturing of NANB hepatitis diagnostic reagents, and development of products such as NANB hepatitis vaccines.

According to the well-known method described by Merrifield, NAMB peptides can be synthesized. Furthermore, the polynucleotides in sequence lists 2-4 and 7 can be used to express polypeptides in host cells such as *Escherichia coli* by means of genetic engineering technique.

A detection system for antibody against NANB hepatitis virus can be developed using polyvinyl microtiter plates and the sandwich method. For example, 50µl of 5 µg/ml concentration of a NANB peptide can be dispensed in each well of the microtiter plates and incubated overnight at room temperature for consolidation. Th microplate w lls can be washed five times with physiological saline containing 0.05% Tween 20. For overcoating, 100 µl of NaCl buffer containing 30% (v/v) of calf serum and 0.05% Tw en 20 (CS buffer) can b dispens d in each well and discarded after incubation for 30 minutes at room temperature.

For determination of NANB antibodies in sampl s, in the primary reaction, 50µl of the CS buff r containing 30% calf serum and 10 µl of a sample can b dispensed in each microplate well and incubated on a microplate vibrator for one hour at room temperature. After completion of the reaction, microplate wells

can be washed five times in th sam way as pr viously described.

In the secondary r action, as label d antibody 1 ng of hors radish peroxidas lab led anti-human IgG mous monoclonal antibodi s (Fab' fragment: 22G, Institut of Immunology Co., Ltd., Tokyo, Japan) dissolved in 50 µl of calf serum can be dispensed in each microplate well, and incubated on a microplate vibrator for one hour at room temperature. Wells can be washed five times in the same way. After addition of hydrogen peroxide (as substrate) and 50 µl of O-phenylendiamine solution (as color developer) in each well, and after incubation for 30 minutes at room temperature, 50 µl of 4M sulphuric acid can be dispensed in each well to stop further color development and for reading absorbance at 492 nm.

The cut-off level of this assay system can be set by measuring a number of donor samples with normal serum ALT (alanine aminotransferase) value of 34 Karmen unit or below and which tested negative for anti-HCV.

The present invention makes possible detection of NANB hepatitis virus infection which could not be detected by conventional determination methods, and provide NANB hepatitis detection kits capable of highly specific and sensitive detection at an early phase of infection.

These features allow accurate diagnosis of patients at an early stage of the disease and also help to remove at higher rate NANB hepatitis virus carrier bloods through screening test of donor bloods.

Polypeptides and their antibodies under this invention can be utilized for manufacture of vaccines and immunological pharmaceuticals, and structural gene of NANB hepatitis virus provides indispensable tools for detection of polypeptide antigens and antibodies.

Antigen-antibody complexes can be detected by methods known in this art. Specific monoclonal and polyclonal antibodies can be obtained by immunizing such animals as mice, guinea pigs, rabbits, goats and horses with NANB peptides (e.g., bearing NANB hepatitis antigenic epitope).

The present invention is based on studies on isolated virus genome of NANB hepatitis virus named HC-J6 and HC-J8, and is completed by clarification of the full sequence of the nucleotides. The invention makes possible highly specific detection of NANB hepatitis virus and provision of polypeptide, polyclonal antibody and monoclonal antibody to prepare the test system.

Further variations and modifications of the invention will become apparent to those skilled in the art from the foregoing and are intended to be encompassed by the claims appended hereto.

Japanese Priority Applications 287402/91 filed August 9, 1991 and 360441/91 filed on December 5, 1991 are relied on and incorporated by reference. U.S. patent applications serial no. 07/540,604 (filed June 19, 1990), 07/653,090 (filed February 8, 1991) , and 07/712,875 (filed June 11, 1991) are incorporated by reference in their entirety.

Sequence list

Sequence list 1:	whole nucleotides of HC-J6 genome RNA
Sequence list 2:	N-9589 whole nucleotides of cDNA to HC-J6 genome RNA
Sequence list 3:	J6-ø81 nucleotides of clone J6-ø81
Sequence list 4:	J6-ø8 nucleotides of clone J6-ø8
Sequence list 5:	P-J6-3033 whole amino acids of ORF of HC-J6 genome
Sequence list 6:	whole nucleotides of HC-J8 genome RNA
Sequence list 7:	whole nucleotides of cDNA to HC-J8 genome RNA
Sequence list 8:	whole amino acids of a variation of ORF of HC-J8 genome
Sequence list 9:	whole amino acids of a variation of ORF of HC-J8 genome

Claims

1. Recombinant RNA of non-A, non-B hepatitis virus, strain HC-J6, comprising the nucleotide sequence of sequence list 1.

2. Recombinant cDNA of non-A, non-B hepatitis virus, strain HC-J6, comprising the nucleotide sequence of sequence list 2.

3. cDNA clone J6-ø81 comprising the nucleotide sequence of sequence list 3.

4. cDNA clone J6-ø8 comprising the nucleotide sequence of sequence list 4.

5. Amino acid sequence corresponding to recombinant cDNA of non-A, non-B hepatitis virus, strain HC-J6, comprising the amino acid s quenc of sequence list 5.

6. Recombinant RNA of non-A, non-B hepatitis virus, strain HC-J8, comprising the nucleotide sequence of sequence list 6.

7. Recombinant cDNA of non-A, non-B hepatitis virus, strain HC-J8, comprising the nucleotide sequence of sequence list 7.

8. Amino acid sequence corresponding to recombinant cDNA of non-A, non-B hepatitis virus, strain HC-J8, comprising the amino acid sequence of sequence list 8.

9. Amino acid sequence corresponding to recombinant cDNA of non-A, non-B hepatitis virus, strain HC-J8, comprising the amino acid sequence of sequence list 9.

10. A non-A, non-B hepatitis diagnostic test kit for analyzing samples for the presence of antibodies directed against a non-A, non-B hepatitis antigen, comprising an antigen attached to a solid substrate and labeled anti-human immunoglobulin; wherein said antigen is an antigen selected from the antigens contained in sequence lists 5, 8 or 9.

11. A method of detecting antibodies directed against a non-A, non-B hepatitis antigen in a sample, said method comprising:
(a) reacting said sample with an antigen selected from the antigens contained in sequence lists 5, 8 or 9 to form antigen-antibody complexes; and
(b) detecting said antigen-antibody complexes.

12. A non-A, non-B hepatitis specific monoclonal or polyclonal antibody reactive with an antigen, said antigen is an antigen selected from the antigens contained in sequence lists 5, 8 or 9.

13. A method of detecting non-A, non-B hepatitis antigen in a sample, said method comprising:
(a) reacting said sample with the non-A, non-B hepatitis monoclonal or polyclonal antibody according to claim 12 to form antigen-antibody complexes; and
(b) detecting said antigen-antibody complexes.

Fig. 1

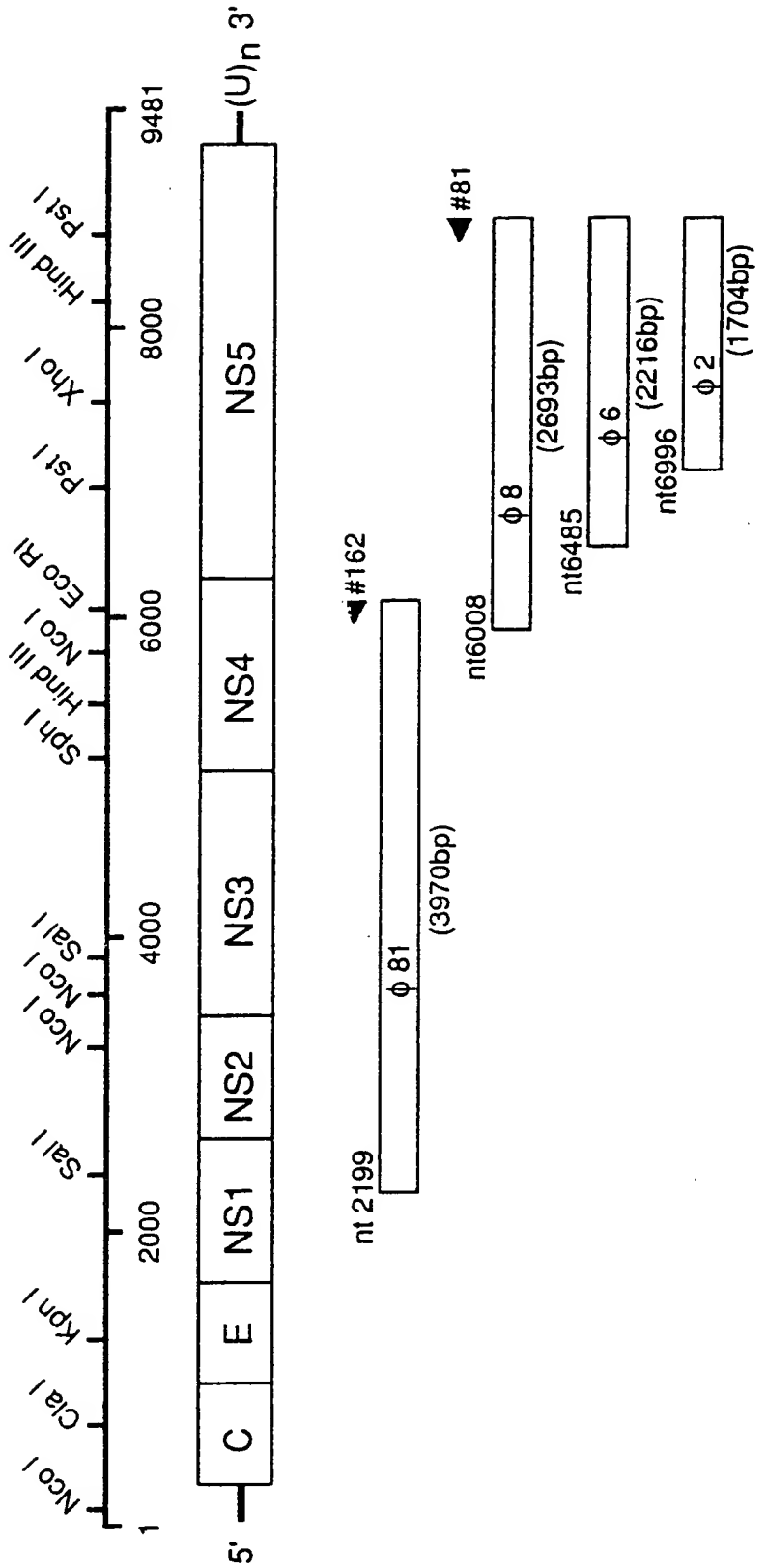
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Fig. 2

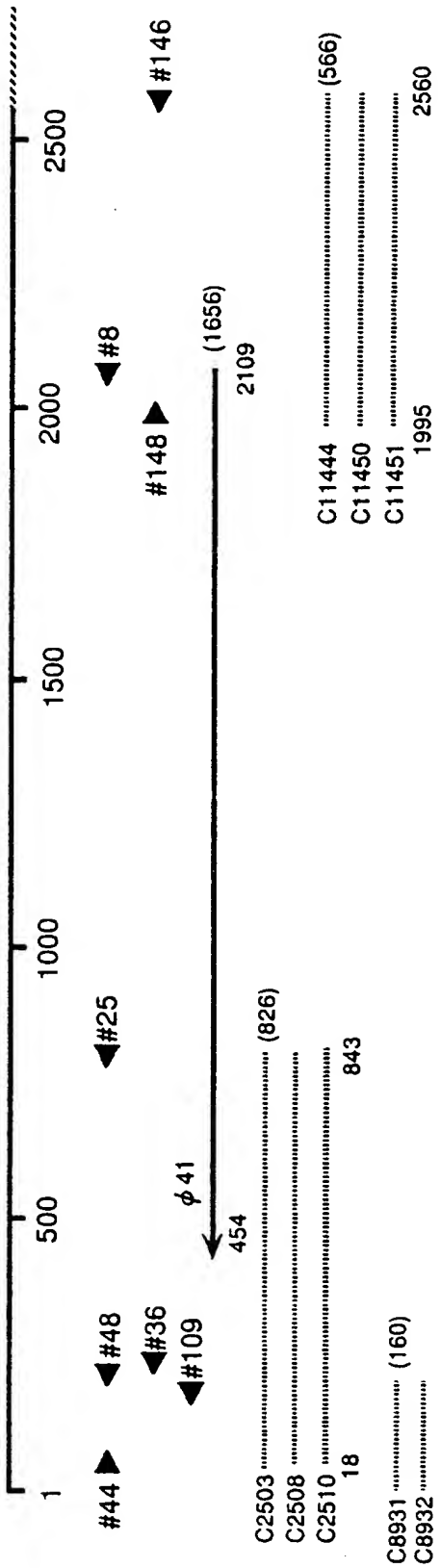
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Fig. 3

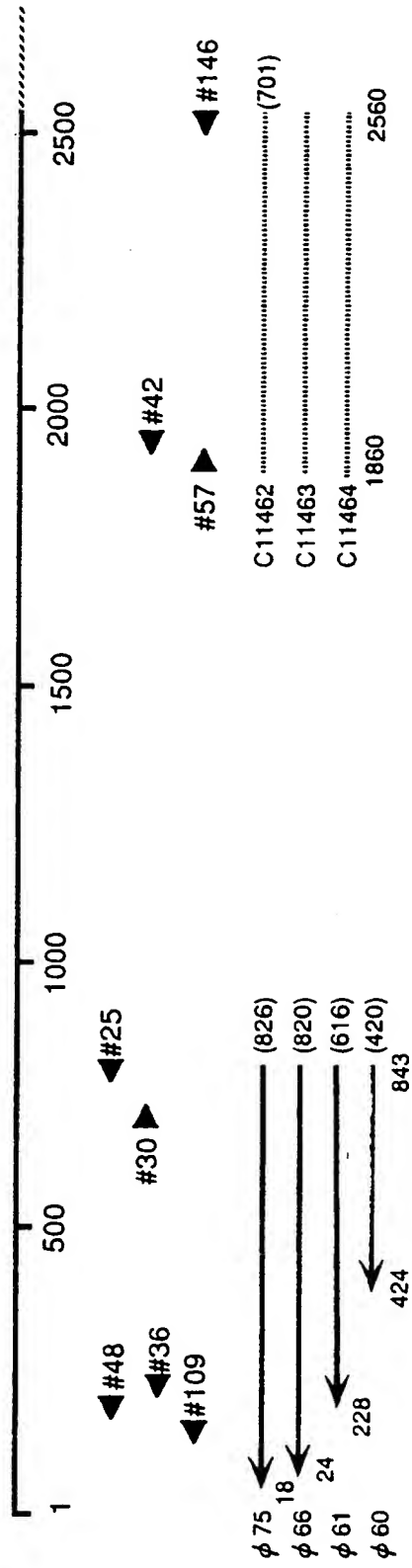
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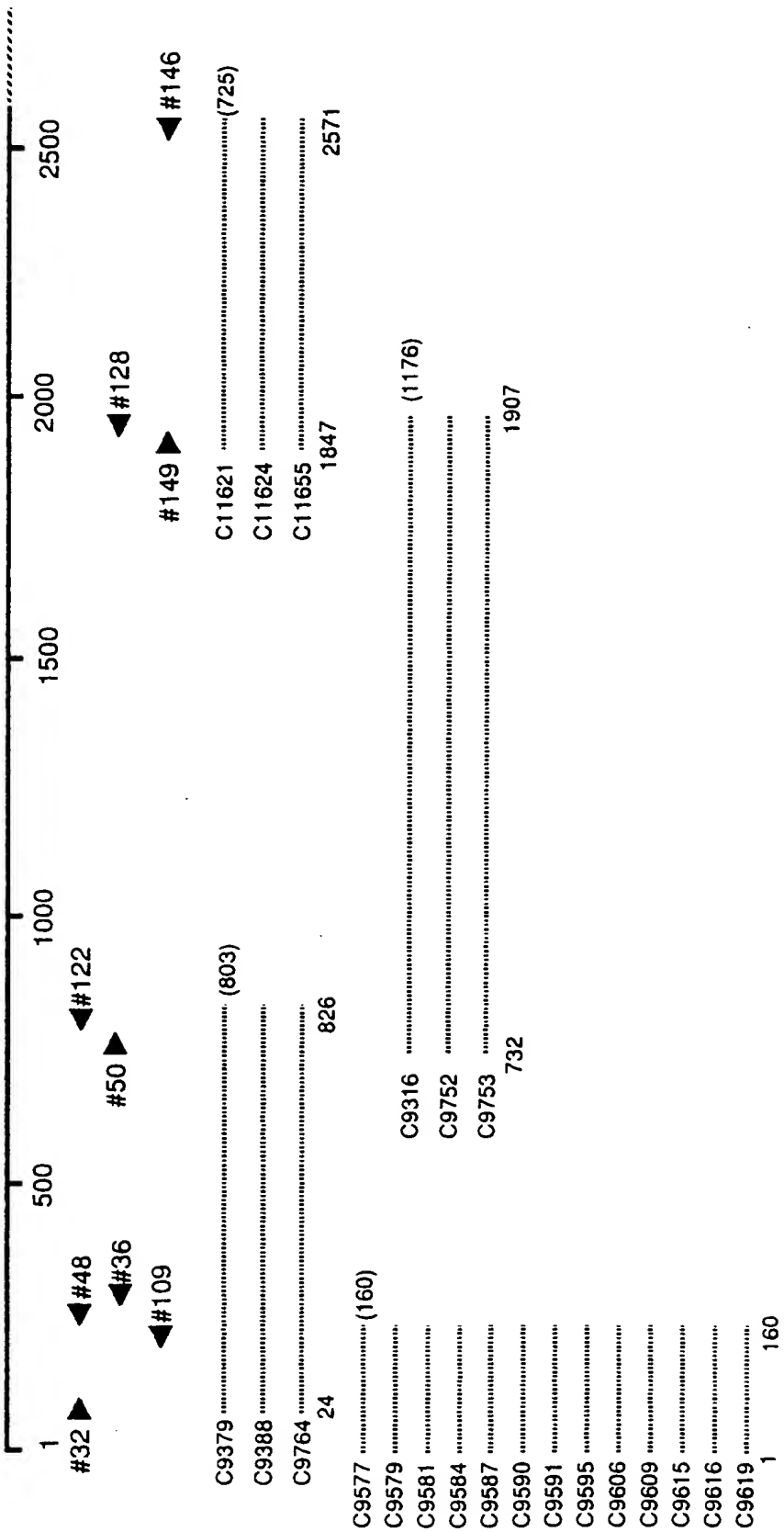
Fig. 4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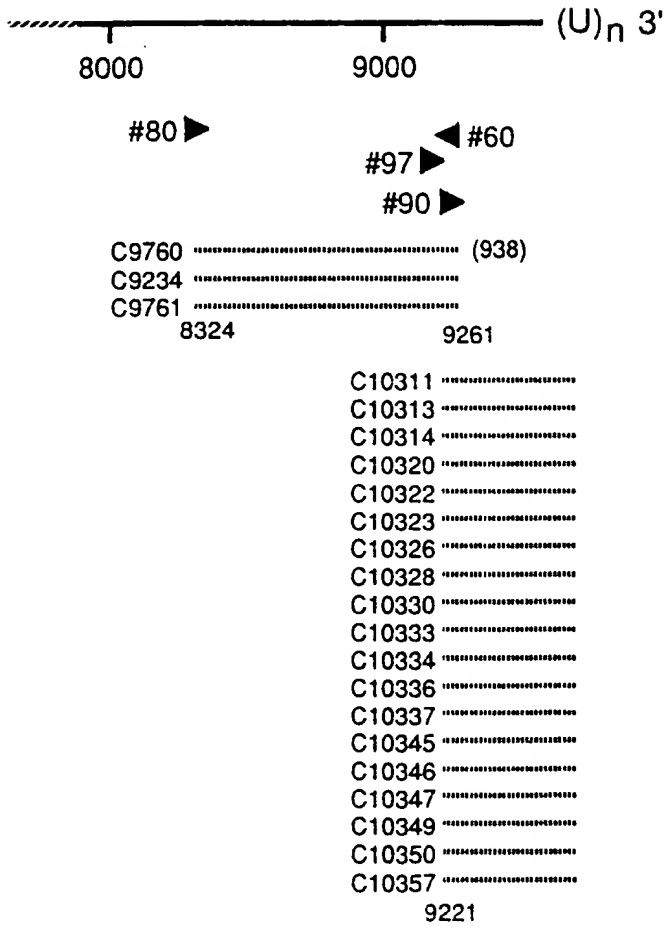
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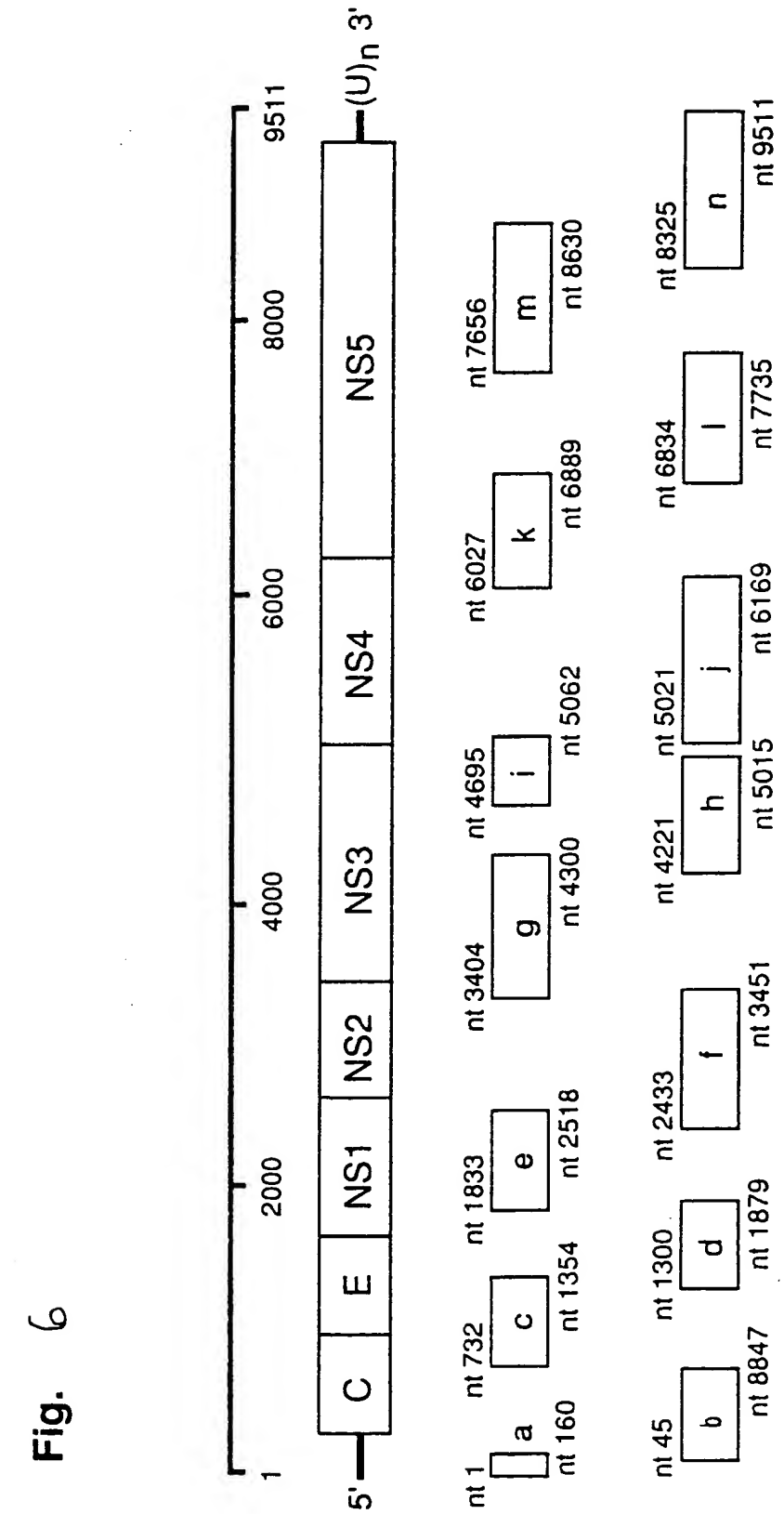
Fig. 6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Molecule Type: cDNA to genomic RNA
Method for Determination of Feature: E

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CTGTCGGGGT CTTCCGGGCA GCCGTGTGCT CCCGGGGCGT GGCCAAGTCC ATAGATTTTA 1740
TCCCCGTTGA GACACTTGAC ATCGTCACTC GGTCCCCCAC CTTTAGTGAC AACAGCACAC 1800
CACCTGCTGT GCCCCAAACT TATCAGGTCG GGTACTTACA TGCCCCGACT GGTAGTGGAA 1860
AGAGCACCAA AGTCCCTGTC GCGTATGCCG CTCAGGGGTA CAAAGTGCTA GTGCTTAATC 1920
CCTCGGTGGC TGCCACCCTG GGGTTTGGGG CGTACTTGTC CAAGGCACAT GGCATCAATC 1980
CCAACATTAG GACTGGGGTC AGGACTGTGA CGACCGGGGC GCCCATCACG TACTCCACAT 2040
ATGGCAAATT CCTCGCCGAT GGGGGCTGCG CAGGCGGCGC CTATGACATC ATCATATGCG 2100
ATGAATGCCA TGCCGTGGAC TCTACCACCA TTCTCGGCAT CGGAACAGTC CTCGATCAAG 2160
CAGAGACAGC CGGGGTCAGG CTAACTGTAC TGGCTACGGC TACGCCCCCC GGGTCAGTGA 2220
CAACCCCCCA CCCCAACATA GAGGAGGTGG CCCTCGGGCA GGAGGGTGAG ATCCCCTTCT 2280
ATGGGAGGGC GATTCCCCTG TCATACATCA AGGGAGGAAG ACACTTGATC TTCTGCCACT 2340
CAAAGAAAAA GTGTGACGAG CTCGCGGCGG CCCTTCGGGG TATGGGCTTG AACGCAGTGG 2400
CATACTACAG AGGGCTGGAC GTCTCCGTAA TACCAACTCA GGGAGACGTA GTGGTCGTCG 2460
CCACCGACGC CCTCATGACG GGGTTTACTG GAGACTTTGA CTCCGTGATC GACTGCAACG 2520
TAGCGGTCAC TCAAGTTGTA GACTTCAGCT TGGACCCCAC ATTCACCATA ACCACACAGA 2580
CTGTCCCTCA AGACGCTGTC TCACGTAGCC AGCGCCGGGG CCGCACGGGC AGGGGAAGAC 2640
TGGGTATTTA TAGGTATGTT TCCACTGGTG AGCGAGCCTC AGGAATGTTT GACAGTGTAG 2700
TGCTCTGCGA GTGCTACGAT GCAGGGGCCG CATGGTATGA GCTCACACCA GCGGAGACCA 2760
CCGTCAGGCT CAGAGCATAT TTCAACACAC CTGGTTTGCC TGTGTGCCAA GACCATCTTG 2820
AGTTTTGGGA GCAGTTTTC ACCGGCCTCA CACACATAGA TGCCCACTTC CTTTCCCAAA 2880
CAAAGCAATC GGGGGAAAAT TTCGCATACT TAACAGCCTA CCAGGCTACA GTGTGCGCTA 2940

GGGCCAAAGC CCCCCCCCCG TCCTGGGACG TCATGTGGAA GTGTTTGACT CGACTCAAGC 3000
CCACACTCGT GGGCCCCACA CCTCTCCTGT ACCGCTTGGG CTCTGTTACC AACGAGGTCA 3060
CCCTCACGCA TCCTGTGACG AAATACATCG CCACCTGCAT GCAAGCCGAC CTTGAGGTCA 3120
TGACCAGCAC GTGGGTCTTA GCTGGGGGGG TCTTGGCGGC CGTCGCCGCG TACTGCCTGG 3180
CGACCGGGTG TGTTTGCATC ATCGGCCGCT TGCACGTTAA CCAGCGAGCC GTCGTTGCAC 3240
CGGACAAGGA GGTCCTCTAT GAGGCTTTTG ATGAGATGGA GGAATGTGCC TCTAGAGCGG 3300
CTCTCATTGA AGAGGGGCAG CGGATAGCCG AGATGCTGAA GTCCAAGATC CAAGGCTTAT 3360
TGCAGCAAGC TTCCAAACAA GCTCAAGACA TACAACCCGC TGTGCAGGCT TCTTGGCCCA 3420
AGGTAGAGCA ATTCTGGGCC AAACACATGT GGAACTTCAT CAGCGGCATT CAATACCTCG 3480
CAGGACTATC AACACTGCCA GGGAACCCTG CTGTAGCTTC CATGATGGCA TTCAGTGCCG 3540
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GTGCGGGCAT TTCGGGGGCT CTCGTCGCAT TCAAGATCAT GTCTGGCGAG AAGCCCTCCA 3780
TGGAGGATGT TGTCAACCTG CTGCCTGGAA TTCTGTCTCC GGGTGCCCTG GTGGTGGGAG 3840
TCATCTGCGC GGCCATCCTG CGCCGACACG TGGGACCGGG GGAAGGCGCT GTCCAATGGA 3900
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CGGAGTCGGA 3970

Sequence ID No.4
Sequence Length: 2,693
Sequence Type: nucleic acid
Strandedness: single
Topology: linear
Molecule Type: cDNA to genomic RNA
Method for Determination of Feature: E

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GGAAACCACG TCGCCCCCAC CCACTACGTG ACGGAGTCGG ATGCGTCGCA GCGTGTGACC  180
CAACTACTTG GCTCCCTTAC CATAACCAGC CTGCTCAGGA GACTCCACAA CTGGATTACT  240
GAAGACTGCC CCATCCCATG CAGCGGCTCG TGGCTCCGCG ATGTGTGGGA TTGGGTTTGC  300
ACCATCCTAA CAGACTTTAA AAACTGGCTG ACCTCCAAAT TGTTCCCAAA GATGCCTGGT  360
CTCCCCTTTA TCTCTTGTCA AAAGGGGTAC AAGGGCGTGT GGGCTGGCAC TGGTATCATG  420
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CCTGACACAG ACGTATTGAC GTCCATGCTA ACAGACCCAT CCCATATCAC GGCGGAGACT  900
GCAGCGCGGC GTTTGGCACG GGGGTCACCC CCGTCCGAGG CAAGCTCCTC AGCGAGCCAG  960
CTATCGGCAC CATCGCTGCG AGCCACCTGC ACCACCCACG GCAAGGCCTA TGATGTGGAC 1020
ATGGTGGATG CCAACCTGTT CATGGGGGGC GATGTGACCC GGATAGAGTC TGAGTCCAAA 1080
GTGGTCGTTC TGGACTCTCT CGACCCAATG GTCGAAGAAA GGAGCGACCT TGAGCCTTCG 1140
ATACCATCGG AATATATGCT CCCCAAGAAG AGATTCCCAC CAGCCTTACC GGCTTGGGCA 1200
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GCCGATTCCG GCAGTCGGAC GCCCCCCGAT GAGTTGGCCC TTTCGGAGAC AGGTTCCATC 1500
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Sequence ID No.5
Sequence Length: 3,033
Sequence Type: amino acid
Topology: linear
Molecule Type: protein

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            20                  25                  30
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        35                  40                  45
Val Arg Ala Thr Arg Lys Thr Ser Glu Arg Ser Gln Pro Arg Gly
    50                  55                  60
Arg Arg Gln Pro Ile Pro Lys Asp Arg Arg Ser Thr Gly Lys Ser
65                  70                  75
Trp Gly Lys Pro Gly Tyr Pro Trp Pro Leu Tyr Gly Asn Glu Gly
                80                  85                  90
Leu Gly Trp Ala Gly Trp Leu Leu Ser Pro Arg Gly Ser Arg Pro
            95                  100                 105
Ser Trp Gly Pro Asn Asp Pro Arg His Arg Ser Arg Asn Val Gly
        110                 115                 120
Lys Val Ile Asp Thr Leu Thr Cys Gly Phe Ala Asp Leu Met Gly
    125                 130                 135
Tyr Ile Pro Val Val Gly Ala Pro Leu Gly Gly Val Ala Arg Ala
140                 145                 150
Leu Ala His Gly Val Arg Val Leu Glu Asp Gly Val Asn Phe Ala
155                 160                 165
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Thr Gly Asn Leu Pro Gly Cys Ser Phe Ser Ile Phe Leu Leu Ala
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185 190 195
Asn Ile Ser Thr Gly Tyr Met Val Thr Asn Asp Cys Thr Asn Asp
200 205 210
Ser Ile Thr Trp Gln Leu Gln Ala Ala Val Leu His Val Pro Gly
215 220 225
Cys Val Pro Cys Glu Lys Val Gly Asn Thr Ser Arg Cys Trp Ile
230 235 240
Pro Val Ser Pro Asn Val Ala Val Gln Gln Pro Gly Ala Leu Thr
245 250 255
Gln Gly Leu Arg Thr His Ile Asp Met Val Val Met Ser Ala Thr
260 265 270
Leu Cys Ser Ala Leu Tyr Val Gly Asp Leu Cys Gly Gly Val Met
275 280 285
Leu Ala Ala Gln Met Phe Ile Val Ser Pro Gln His His Trp Phe
290 295 300
Val Gln Asp Cys Asn Cys Ser Ile Tyr Pro Gly Thr Ile Thr Gly
305 310 315
His Arg Met Ala Trp Asp Met Met Met Asn Trp Ser Pro Thr Ala
320 325 330
Thr Met Ile Leu Ala Tyr Ala Met Arg Val Pro Glu Val Ile Ile
335 340 345
Asp Ile Ile Gly Gly Ala His Trp Gly Val Met Phe Gly Leu Ala
350 355 360
Tyr Phe Ser Met Gln Gly Ala Trp Ala Lys Val Val Val Ile Leu
365 370 375
Leu Leu Ala Ala Gly Val Asp Ala Gln Thr His Thr Val Gly Gly

380 385 390

Ser Thr Ala His Asn Ala Arg Thr Leu Thr Gly Met Phe Ser Leu

395 400 405

Gly Ala Arg Gln Lys Ile Gln Leu Ile Asn Thr Asn Gly Ser Trp

410 415 420

His Ile Asn Arg Thr Ala Leu Asn Cys Asn Asp Ser Leu His Thr

425 430 435

Gly Phe Leu Ala Ser Leu Phe Tyr Thr His Ser Phe Asn Ser Ser

440 445 450

Gly Cys Pro Glu Arg Met Ser Ala Cys Arg Ser Ile Glu Ala Phe

455 460 465

Arg Val Gly Trp Gly Ala Leu Gln Tyr Glu Asp Asn Val Thr Asn

470 475 480

Pro Glu Asp Met Arg Pro Tyr Cys Trp His Tyr Pro Pro Arg Gln

485 490 495

Cys Gly Val Val Ser Ala Ser Ser Val Cys Gly Pro Val Tyr Cys

500 505 510

Phe Thr Pro Ser Pro Val Val Val Gly Thr Thr Asp Arg Leu Gly

515 520 525

Ala Pro Thr Tyr Thr Trp Gly Glu Asn Glu Thr Asp Val Phe Leu

530 535 540

Leu Asn Ser Thr Arg Pro Pro Gln Gly Ser Trp Phe Gly Cys Thr

545 550 555

Trp Met Asn Ser Thr Gly Tyr Thr Lys Thr Cys Gly Ala Pro Pro

560 565 570

Cys Arg Ile Arg Ala Asp Phe Asn Ala Ser Met Asp Leu Leu Cys

575 580 585

Pro Thr Asp Cys Phe Arg Lys His Pro Asp Thr Thr Tyr Ile Lys

590 595 600

Cys Gly Ser Gly Pro Trp Leu Thr Pro Arg Cys Leu Ile Asp Tyr
605 610 615
Pro Tyr Arg Leu Trp His Tyr Pro Cys Thr Val Asn Tyr Thr Ile
620 625 630
Phe Lys Ile Arg Met Tyr Val Gly Gly Val Glu His Arg Leu Thr
635 640 645
Ala Ala Cys Asn Phe Thr Arg Gly Asp Arg Cys Asn Leu Glu Asp
650 655 660
Arg Asp Arg Ser Gln Leu Ser Pro Leu Leu His Ser Thr Thr Glu
665 670 675
Trp Ala Ile Leu Pro Cys Thr Tyr Ser Asp Leu Pro Ala Leu Ser
680 685 690
Thr Gly Leu Leu His Leu His Gln Asn Ile Val Asp Val Gln Phe
695 700 705
Met Tyr Gly Leu Ser Pro Ala Leu Thr Lys Tyr Ile Val Arg Trp
710 715 720
Glu Trp Val Val Leu Leu Phe Leu Leu Leu Ala Asp Ala Arg Val
725 730 735
Cys Ala Cys Leu Trp Met Leu Ile Leu Leu Gly Gln Ala Glu Ala
740 745 750
Ala Leu Glu Lys Leu Val Val Leu His Ala Ala Ser Ala Ala Ser
755 760 765
Cys Asn Gly Phe Leu Tyr Phe Val Ile Phe Phe Val Ala Ala Trp
770 775 780
Tyr Ile Lys Gly Arg Val Val Pro Leu Ala Thr Tyr Ser Leu Thr
785 790 795
Gly Leu Trp Ser Phe Gly Leu Leu Leu Leu Ala Leu Pro Gln Gln
800 805 810
Ala Tyr Ala Tyr Asp Ala Ser Val His Gly Gln Ile Gly Ala Ala

815 820 825

Leu Leu Val Leu Ile Thr Leu Phe Thr Leu Thr Pro Gly Tyr Lys

830 835 840

Thr Leu Leu Ser Arg Phe Leu Trp Trp Leu Cys Tyr Leu Leu Thr

845 850 855

Leu Ala Glu Ala Met Val Gln Glu Trp Ala Pro Pro Met Gln Val

860 865 870

Arg Gly Gly Arg Asp Gly Ile Ile Trp Ala Val Ala Ile Phe Cys

875 880 885

Pro Gly Val Val Phe Asp Ile Thr Lys Trp Leu Leu Ala Val Leu

890 895 900

Gly Pro Ala Tyr Leu Leu Lys Gly Ala Leu Thr Arg Val Pro Tyr

905 910 915

Phe Val Arg Ala His Ala Leu Leu Arg Met Cys Thr Met Val Arg

920 925 930

His Leu Ala Gly Gly Arg Tyr Val Gln Met Val Leu Leu Ala Leu

935 940 945

Gly Arg Trp Thr Gly Thr Tyr Ile Tyr Asp His Leu Thr Pro Met

950 955 960

Ser Asp Trp Ala Ala Asn Gly Leu Arg Asp Leu Ala Val Ala Val

965 970 975

Glu Pro Ile Ile Phe Ser Pro Met Glu Lys Lys Val Ile Val Trp

980 985 990

Gly Ala Glu Thr Ala Ala Cys Gly Asp Ile Leu His Gly Leu Pro

995 1000 1005

Val Ser Ala Arg Leu Gly Arg Glu Val Leu Leu Gly Pro Ala Asp

1010 1015 1020

Gly Tyr Thr Ser Lys Gly Trp Ser Leu Leu Ala Pro Ile Thr Ala

1025 1030 1035

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1040 1045 1050
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1055 1060 1065
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1070 1075 1080
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1100 1105 1110
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1115 1120 1125
Pro Cys Thr Cys Gly Ala Val Asp Leu Tyr Leu Val Thr Arg Asn
1130 1135 1140
Ala Asp Val Ile Pro Ala Arg Arg Arg Gly Asp Lys Arg Gly Ala
1145 1150 1155
Leu Leu Ser Pro Arg Pro Leu Ser Thr Leu Lys Gly Ser Ser Gly
1160 1165 1170
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1175 1180 1185
Ala Ala Val Cys Ser Arg Gly Val Ala Lys Ser Ile Asp Phe Ile
1190 1195 1200
Pro Val Glu Thr Leu Asp Ile Val Thr Arg Ser Pro Thr Phe Ser
1205 1210 1215
Asp Asn Ser Thr Pro Pro Ala Val Pro Gln Thr Tyr Gln Val Gln
1220 1225 1230
Tyr Leu His Ala Pro Thr Gly Ser Gly Lys Ser Thr Lys Val Pro
1235 1240 1245
Val Ala Tyr Ala Ala Gln Gly Tyr Lys Val Leu Val Leu Asn Pro

1250 1255 1260

Ser Val Ala Ala Thr Leu Gly Phe Gly Ala Tyr Leu Ser Lys Ala

1265 1270 1275

His Gly Ile Asn Pro Asn Ile Arg Thr Gly Val Arg Thr Val Thr

1280 1285 1290

Thr Gly Ala Pro Ile Thr Tyr Ser Thr Tyr Gly Lys Phe Leu Ala

1295 1300 1305

Asp Gly Gly Cys Ala Gly Gly Ala Tyr Asp Ile Ile Ile Cys Asp

1310 1315 1320

Glu Cys His Ala Val Asp Ser Thr Thr Ile Leu Gly Ile Gly Thr

1325 1330 1335

Val Leu Asp Gln Ala Glu Thr Ala Gly Val Arg Leu Thr Val Leu

1340 1345 1350

Ala Thr Ala Thr Pro Pro Gly Ser Val Thr Thr Pro His Pro Asn

1355 1360 1365

Ile Glu Glu Val Ala Leu Gly Gln Glu Gly Glu Ile Pro Phe Tyr

1370 1375 1380

Gly Arg Ala Ile Pro Leu Ser Tyr Ile Lys Gly Gly Arg His Leu

1385 1390 1395

Ile Phe Cys His Ser Lys Lys Lys Cys Asp Glu Leu Ala Ala Ala

1400 1405 1410

Leu Arg Gly Met Gly Leu Asn Ala Val Ala Tyr Tyr Arg Gly Leu

1415 1420 1425

Asp Val Ser Val Ile Pro Thr Gln Gly Asp Val Val Val Val Ala

1430 1435 1440

Thr Asp Ala Leu Met Thr Gly Phe Thr Gly Asp Phe Asp Ser Val

1445 1450 1455

Ile Asp Cys Asn Val Ala Val Thr Gln Val Val Asp Phe Ser Leu

1460 1465 1470

Asp Pro Thr Phe Thr Ile Thr Thr Gln Thr Val Pro Gln Asp Ala
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1490 1495 1500
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1535 1540 1545
Tyr Phe Asn Thr Pro Gly Leu Pro Val Cys Gln Asp His Leu Glu
1550 1555 1560
Phe Trp Glu Ala Val Phe Thr Gly Leu Thr His Ile Asp Ala His
1565 1570 1575
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1580 1585 1590
Thr Ala Tyr Gln Ala Thr Val Cys Ala Arg Ala Lys Ala Pro Pro
1595 1600 1605
Pro Ser Trp Asp Val Met Trp Lys Cys Leu Thr Arg Leu Lys Pro
1610 1615 1620
Trp Leu Val Gly Pro Thr Pro Leu Leu Tyr Arg Leu Gly Ser Val
1625 1630 1635
Thr Asn Glu Val Thr Leu Thr His Pro Val Thr Lys Tyr Ile Ala
1640 1645 1650
Thr Cys Met Gln Ala Asp Leu Glu Val Met Thr Ser Thr Trp Val
1655 1660 1665
Leu Ala Gly Gly Val Leu Ala Ala Val Ala Ala Tyr Cys Leu Ala
1670 1675 1680
Thr Gly Cys Val Cys Ile Ile Gly Arg Leu His Val Asn Gln Arg

1685 1690 1695

Ala Val Val Ala Pro Asp Lys Glu Val Leu Tyr Glu Ala Phe Asp

1700 1705 1710

Glu Met Glu Glu Cys Ala Ser Arg Ala Ala Leu Ile Glu Glu Gly

1715 1720 1725

Gln Arg Ile Ala Glu Met Leu Lys Ser Lys Ile Gln Gly Leu Leu

1730 1735 1740

Gln Gln Ala Ser Lys Gln Ala Gln Asp Ile Gln Pro Ala Val Gln

1745 1750 1755

Ala Ser Trp Pro Lys Val Glu Gln Phe Trp Ala Lys His Met Trp

1760 1765 1770

Asn Phe Ile Ser Gly Ile Gln Tyr Leu Ala Gly Leu Ser Thr Leu

1775 1780 1785

Pro Gly Asn Pro Ala Val Ala Ser Met Met Ala Phe Ser Ala Ala

1790 1795 1800

Leu Thr Ser Pro Leu Ser Thr Ser Thr Thr Ile Leu Leu Asn Ile

1805 1810 1815

Leu Gly Gly Trp Leu Ala Ser Gln Ile Ala Pro Pro Ala Gly Ala

1820 1825 1830

Thr Gly Phe Val Val Ser Gly Leu Val Gly Ala Ala Val Gly Ser

1835 1840 1845

Ile Gly Leu Gly Lys Val Leu Val Asp Ile Leu Ala Gly Tyr Gly

1850 1855 1860

Ala Gly Ile Ser Gly Ala Leu Val Ala Phe Lys Ile Met Ser Gly

1865 1870 1875

Glu Lys Pro Ser Met Glu Asp Val Val Asn Leu Leu Pro Gly Ile

1880 1885 1890

Leu Ser Pro Gly Ala Leu Val Val Gly Val Ile Cys Ala Ala Ile

1895 1900 1905

Leu Arg Arg His Val Gly Pro Gly Glu Gly Ala Val Gln Trp Met
1910 1915 1920
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1925 1930 1935
Thr His Tyr Val Thr Glu Ser Asp Ala Ser Gln Arg Val Thr Gln
1940 1945 1950
Leu Leu Gly Ser Leu Thr Ile Thr Ser Leu Leu Arg Arg Leu His
1955 1960 1965
Asn Trp Ile Thr Glu Asp Cys Pro Ile Pro Cys Ser Gly Ser Trp
1970 1975 1980
Leu Arg Asp Val Trp Asp Trp Val Cys Thr Ile Leu Thr Asp Phe
1985 1990 1995
Lys Asn Trp Leu Thr Ser Lys Leu Phe Pro Lys Met Pro Gly Leu
2000 2005 2010
Pro Phe Ile Ser Cys Gln Lys Gly Tyr Lys Gly Val Trp Ala Gly
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2075 2080 2085
Ile Trp Arg Val Ala Ala Ser Glu Tyr Ala Glu Val Thr Gln His
2090 2095 2100
Gly Ser Tyr His Tyr Ile Thr Gly Leu Thr Thr Asp Asn Leu Lys
2105 2110 2115
Val Pro Cys Gln Leu Pro Ser Pro Glu Phe Phe Ser Trp Val Asp

2120 2125 2130

Gly Val Gln Ile His Arg Phe Ala Pro Ile Pro Lys Pro Phe Phe

2135 2140 2145

Arg Asp Glu Val Ser Phe Cys Val Gly Leu Asn Ser Phe Val Val

2150 2155 2160

Gly Ser Gln Leu Pro Cys Asp Pro Glu Pro Asp Thr Asp Val Leu

2165 2170 2175

Thr Ser Met Leu Thr Asp Pro Ser His Ile Thr Ala Glu Thr Ala

2180 2185 2190

Ala Arg Arg Leu Ala Arg Gly Ser Pro Pro Ser Glu Ala Ser Ser

2195 2200 2205

Ser Ala Ser Gln Leu Ser Ala Pro Ser Leu Arg Ala Thr Cys Thr

2210 2215 2220

Thr His Gly Lys Ala Tyr Asp Val Asp Met Val Asp Ala Asn Leu

2225 2230 2235

Phe Met Gly Gly Asp Val Thr Arg Ile Glu Ser Glu Ser Lys Val

2240 2245 2250

Val Val Leu Asp Ser Leu Asp Pro Met Val Glu Glu Arg Ser Asp

2255 2260 2265

Leu Glu Pro Ser Ile Pro Ser Glu Tyr Met Leu Pro Lys Lys Arg

2270 2275 2280

Phe Pro Pro Ala Leu Pro Ala Trp Ala Arg Pro Asp Tyr Asn Pro

2285 2290 2295

Pro Leu Val Glu Ser Trp Lys Arg Pro Asp Tyr Gln Pro Ala Thr

2300 2305 2310

Val Ala Gly Cys Ala Leu Pro Pro Pro Lys Lys Thr Pro Thr Pro

2315 2320 2325

Pro Pro Arg Arg Arg Arg Thr Val Gly Leu Ser Glu Ser Ser Ile

2330 2335 2340

Ala Asp Ala Leu Gln Gln Leu Ala Ile Lys Ser Phe Gly Gln Pro
2345 2350 2355
Pro Pro Ser Gly Asp Ser Gly Leu Ser Thr Gly Ala Asp Ala Ala
2360 2365 2370
Asp Ser Gly Ser Arg Thr Pro Pro Asp Glu Leu Ala Leu Ser Glu
2375 2380 2385
Thr Gly Ser Ile Ser Ser Met Pro Pro Leu Glu Gly Glu Pro Gly
2390 2395 2400
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2435 2440 2445
Tyr Ser Trp Thr Gly Ala Leu Ile Thr Pro Cys Ser Pro Glu Glu
2450 2455 2460
Glu Lys Leu Pro Ile Asn Pro Leu Ser Asn Ser Leu Leu Arg Tyr
2465 2470 2475
His Asn Lys Val Tyr Cys Thr Thr Ser Lys Ser Ala Ser Leu Arg
2480 2485 2490
Ala Lys Lys Val Thr Phe Asp Arg Met Gln Ala Leu Asp Ala His
2495 2500 2505
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2525 2530 2535
Pro His Ser Ala Arg Ser Lys Tyr Gly Phe Gly Ala Lys Glu Val
2540 2545 2550

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Ser Leu Pro Glu Glu Ala His Thr Ala Ile His Ser Leu Thr Glu
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Arg Leu Tyr Val Gly Gly Pro Met Phe Asn Ser Lys Gly Gln Thr
2705 2710 2715
Cys Gly Tyr Arg Arg Cys Arg Ala Ser Gly Val Leu Thr Thr Ser
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Met Gly Asn Thr Ile Thr Cys Tyr Val Lys Ala Leu Ala Ala Cys
2735 2740 2745
Lys Ala Ala Gly Ile Ile Ala Pro Thr Met Leu Val Cys Gly Asp
2750 2755 2760

Asp Leu Val Val Ile Ser Glu Ser Gln Gly Thr Glu Glu Asp Glu
2765 2770 2775
Arg Asn Leu Arg Ala Phe Thr Glu Ala Met Thr Arg Tyr Ser Ala
2780 2785 2790
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2825 2830 2835
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2840 2845 2850
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Val Leu Met Thr His Phe Phe Ser Ile Leu Met Ala Gln Asp Thr
2870 2875 2880
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2885 2890 2895
Val Ser Pro Leu Asp Leu Pro Ala Ile Ile Glu Arg Leu His Gly
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2930 2935 2940
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2945 2950 2955
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2960 2965 2970

Ala Val Lys Thr Lys Leu Lys Leu Thr Pro Leu Pro Glu Ala Arg
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2990 2995 3000
Asp Ile Tyr His Ser Val Ser Arg Ala Arg Pro Arg Leu Leu Leu
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3033

Sequence ID No. 6
Sequence Length: 9,511
Sequence Type: nucleic acid
Strandedness: single
Topology: linear
Molecule Type: genomic RNA
Method for Determination of Feature: E

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Sequence ID No. 7
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Strandedness: single
Topology: linear
Molecule Type: cDNA to genomic RNA
Method for Determination of Feature: E

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Sequence ID No. 3
Sequence Length: 3,033
Sequence Type: amino acid
Topology: linear
Molecule Type: protein

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 35                  40                  45
Val Arg Ala Thr Arg Lys Thr Ser Glu Arg Ser Gln Pro Arg Gly
 50                  55                  60
Arg Arg Gln Pro Ile Pro Lys Asp Arg Arg Ser Thr Gly Lys Ser
 65                  70                  75
Trp Gly Lys Pro Gly Tyr Pro Trp Pro Leu Tyr Gly Asn Glu Gly
 80                  85                  90
Cys Gly Trp Ala Gly Trp Leu Leu Ser Pro Arg Gly Ser Arg Pro
 95                 100                 105
Thr Trp Gly Pro Thr Asp Pro Arg His Arg Ser Arg Asn Leu Gly
110                 115                 120
Arg Val Ile Asp Thr Ile Thr Cys Gly Phe Ala Asp Leu Met Gly
125                 130                 135
Tyr Ile Pro Val Val Gly Ala Pro Val Gly Gly Val Ala Arg Ala
140                 145                 150
Leu Ala His Gly Val Arg Val Leu Glu Asp Gly Ile Asn Tyr Ala
155                 160                 165
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215 220 225
Cys Val Pro Cys Glu Asn Asp Asn Gly Thr Leu His Cys Trp Ile
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Gln Val Thr Pro Asn Val Ala Val Lys His Arg Gly Ala Leu Thr
245 250 255
Arg Ser Leu Arg Thr His Val Asp Met Ile Val Met Ala Ala Thr
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Ala Cys Ser Ala Leu Tyr Val Gly Asp Val Cys Gly Ala Val Met
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Ile Leu Ser Gln Ala Phe Met Val Ser Pro Gln Arg His Asn Phe
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Thr Gln Glu Cys Asn Cys Ser Ile Tyr Gln Gly His Ile Thr Gly
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His Arg Met Ala Trp Asp Met Met Leu Ser Trp Ser Pro Thr Leu
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335 340 345
Glu Ile Ile Phe Gly Gly His Trp Gly Val Val Phe Gly Leu Ala
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Tyr Phe Ser Met Gln Gly Ala Trp Ala Lys Val Ile Ala Ile Leu
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Gly Phe Leu Ala Ser Leu Phe Tyr Thr His Lys Phe Asn Ser Ser
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Gly Cys Pro Glu Arg Leu Ser Ser Cys Arg Gly Leu Asp Asp Phe
                455                 460                 465
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                470                 475                 480
Asp Gly Asp Met Arg Pro Tyr Cys Trp His Tyr Pro Pro Arg Pro
                485                 490                 495
Cys Gly Ile Val Pro Ala Arg Thr Val Cys Gly Pro Val Tyr Cys
                500                 505                 510
Phe Thr Pro Ser Pro Val Val Val Gly Thr Thr Asp Lys Gln Gly
                515                 520                 525
Val Pro Thr Tyr Thr Trp Gly Glu Asn Glu Thr Asp Val Phe Leu
                530                 535                 540
Leu Asn Ser Thr Arg Pro Pro Arg Gly Ala Trp Phe Gly Cys Thr
                545                 550                 555
Trp Met Asn Gly Thr Gly Phe Thr Lys Thr Cys Gly Ala Pro Pro
                560                 565                 570
Cys Arg Ile Arg Lys Asp Tyr Asn Ser Thr Ile Asp Leu Leu Cys
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Pro Thr Asp Cys Phe Arg Lys His Pro Asp Ala Thr Tyr Leu Lys
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Pro Tyr Arg Leu Trp His Tyr Pro Cys Thr Val Asn Phe Thr Ile
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Phe Lys Ala Arg Met Tyr Val Gly Gly Val Glu His Arg Phe Ser
635 640 645
Ala Ala Cys Asn Phe Thr Arg Gly Asp Arg Cys Arg Leu Glu Asp
650 655 660
Arg Asp Arg Gly Gln Gln Ser Pro Leu Leu His Ser Thr Thr Glu
665 670 675
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680 685 690
Thr Gly Leu Leu His Leu His Gln Asn Ile Val Asp Val Gln Tyr
695 700 705
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Glu Trp Val Ile Leu Leu Phe Leu Leu Leu Ala Asp Ala Arg Ile
725 730 735
Cys Ala Cys Leu Trp Met Leu Ile Ile Leu Gly Gln Ala Glu Ala
740 745 750
Ala Leu Glu Lys Leu Ile Ile Leu His Ser Ala Ser Ala Ala Ser
755 760 765
Ala Asn Gly Pro Leu Trp Phe Phe Ile Phe Phe Thr Ala Ala Trp
770 775 780
Tyr Leu Lys Gly Arg Val Val Pro Val Ala Thr Tyr Ser Val Leu
785 790 795
Gly Leu Trp Ser Phe Leu Leu Leu Val Leu Ala Leu Pro Gln Gln
800 805 810
Ala Tyr Ala Leu Asp Ala Ala Glu Gln Gly Glu Leu Gly Leu Ala

815 820 825

Ile Leu Val Ile Ile Ser Ile Phe Thr Leu Thr Pro Ala Tyr Lys

830 835 840

Ile Leu Leu Ser Arg Ser Val Trp Trp Leu Ser Tyr Met Leu Val

845 850 855

Leu Ala Glu Ala Gln Ile Gln Gln Trp Val Pro Pro Leu Glu Val

860 865 870

Arg Gly Gly Arg Asp Gly Ile Ile Trp Val Ala Val Ile Leu His

875 880 885

Pro Arg Leu Val Phe Glu Val Thr Lys Trp Leu Leu Ala Ile Leu

890 895 900

Gly Pro Ala Tyr Leu Leu Lys Ala Ser Leu Leu Arg Ile Pro Tyr

905 910 915

Phe Val Arg Ala His Ala Leu Leu Arg Val Cys Thr Leu Val Lys

920 925 930

His Leu Ala Gly Ala Arg Tyr Ile Gln Met Leu Leu Ile Thr Ile

935 940 945

Gly Arg Trp Thr Gly Thr Tyr Ile Tyr Asp His Leu Ser Pro Leu

950 955 960

Ser Thr Trp Ala Ala Gln Gly Leu Arg Asp Leu Ala Ile Ala Val

965 970 975

Glu Pro Val Val Phe Ser Pro Met Glu Lys Lys Val Ile Val Trp

980 985 990

Gly Ala Glu Thr Val Ala Cys Gly Asp Ile Leu His Gly Leu Pro

995 1000 1005

Val Ser Ala Arg Leu Gly Arg Glu Val Leu Leu Gly Pro Ala Asp

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1190 1195 1200
Pro Val Glu Ser Leu Asp Val Ala Thr Arg Thr Pro Ser Phe Ser
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His Gly Ile Asn Pro Asn Ile Arg Thr Gly Val Arg Thr Val Thr

1280 1285 1290

Thr Gly Asp Ser Ile Thr Tyr Ser Thr Tyr Gly Lys Phe Ile Ala

1295 1300 1305

Asp Gly Gly Cys Ala Ala Gly Ala Tyr Asp Ile Ile Ile Cys Asp

1310 1315 1320

Glu Cys His Ser Val Asp Ala Thr Thr Ile Leu Gly Ile Gly Thr

1325 1330 1335

Val Leu Asp Gln Ala Glu Thr Ala Gly Val Arg Leu Val Val Leu

1340 1345 1350

Ala Thr Ala Thr Pro Pro Gly Thr Val Thr Thr Pro His Ser Asn

1355 1360 1365

Ile Glu Glu Val Ala Leu Gly His Glu Gly Glu Ile Pro Phe Tyr

1370 1375 1380

Gly Lys Ala Ile Pro Leu Ala Phe Ile Lys Gly Gly Arg His Leu

1385 1390 1395

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Thr Asp Ala Leu Met Thr Gly Tyr Thr Gly Asp Phe Asp Ser Val

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1685 1690 1695

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1700 1705 1710

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1715 1720 1725

Gln Arg Met Ala Glu Met Leu Lys Ser Lys Ile Gln Gly Leu Leu

1730 1735 1740

Gln Gln Ala Thr Arg Gln Ala Gln Asp Ile Gln Pro Ala Ile Gln

1745 1750 1755

Ser Ser Trp Pro Lys Leu Glu Gln Phe Trp Ala Lys His Met Trp

1760 1765 1770

Asn Phe Ile Ser Gly Ile Gln Tyr Leu Ala Gly Leu Ser Thr Leu

1775 1780 1785

Pro Gly Asn Pro Ala Val Ala Ser Met Met Ala Phe Ser Ala Ala

1790 1795 1800

Leu Thr Ser Pro Leu Pro Thr Ser Thr Thr Ile Leu Leu Asn Ile

1805 1810 1815

Met Gly Gly Trp Leu Ala Ser Gln Ile Ala Pro Pro Ala Gly Ala

1820 1825 1830

Thr Gly Phe Val Val Ser Gly Leu Val Gly Ala Ala Val Gly Ser

1835 1840 1845

Ile Gly Leu Gly Lys Ile Leu Val Asp Val Leu Ala Gly Tyr Gly

1850 1855 1860

Ala Gly Ile Ser Gly Ala Leu Val Ala Phe Lys Ile Met Ser Gly

1865 1870 1875

Glu Lys Pro Thr Val Glu Asp Val Val Asn Leu Leu Pro Ala Ile

1880 1885 1890

Leu Ser Pro Gly Ala Leu Val Val Gly Val Ile Cys Ala Ala Ile

1895 1900 1905

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1925 1930 1935
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1940 1945 1950
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1955 1960 1965
Ala Trp Ile Thr Glu Asp Cys Pro Val Pro Cys Ser Gly Ser Trp
1970 1975 1980
Leu Gln Asp Ile Trp Asp Trp Val Cys Ser Ile Leu Thr Asp Phe
1985 1990 1995
Lys Asn Trp Leu Ser Ser Lys Leu Leu Pro Lys Met Pro Gly Ile
2000 2005 2010
Pro Phe Ile Ser Cys Gln Lys Gly Tyr Lys Gly Val Trp Ala Gly
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2135 2140 2145

Arg Asp Glu Val Thr Phe Thr Val Gly Leu Asn Ser Phe Val Val

2150 2155 2160

Gly Ser Gln Leu Pro Cys Asp Pro Glu Pro Asp Thr Glu Val Leu

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2195 2200 2205

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Thr His Lys Thr Ala Tyr Asp Cys Asp Met Val Asp Ala Asn Leu

2225 2230 2235

Phe Met Gly Gly Asp Val Thr Arg Ile Glu Ser Asp Ser Lys Val

2240 2245 2250

Ile Val Leu Asp Ser Leu Asp Ser Met Thr Glu Val Glu Asp Asp

2255 2260 2265

Arg Glu Pro Ser Val Pro Ser Glu Tyr Leu Ile Lys Arg Arg Lys

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2315 2320 2325

Pro Pro Arg Arg Arg Arg Ala Lys Val Leu Thr Gln Asp Asn Val

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2375 2380 2385
Ala Gly Ser Leu Ser Ser Met Pro Pro Leu Glu Gly Glu Pro Gly
2390 2395 2400
Asp Pro Asp Leu Glu Phe Glu Pro Val Gly Ser Ala Pro Pro Ser
2405 2410 2415
Glu Gly Glu Cys Glu Val Ile Asp Ser Asp Ser Lys Ser Trp Ser
2420 2425 2430
Thr Val Ser Asp Gln Glu Asp Ser Val Ile Cys Cys Ser Met Ser
2435 2440 2445
Tyr Ser Trp Thr Gly Ala Leu Ile Thr Pro Cys Gly Pro Glu Glu
2450 2455 2460
Glu Lys Leu Pro Ile Asn Pro Leu Ser Asn Ser Leu Met Arg Phe
2465 2470 2475
His Asn Lys Val Tyr Ser Thr Thr Ser Arg Ser Ala Ser Leu Arg
2480 2485 2490
Ala Lys Lys Val Thr Phe Asp Arg Val Gln Val Leu Asp Ala His
2495 2500 2505
Tyr Asp Ser Val Leu Gln Asp Val Lys Arg Ala Ala Ser Lys Val
2510 2515 2520
Ser Ala Arg Leu Leu Thr Val Glu Glu Ala Cys Ala Leu Thr Pro
2525 2530 2535
Pro His Ser Ala Lys Ser Arg Tyr Gly Phe Gly Ala Lys Glu Val
2540 2545 2550
Arg Ser Leu Ser Arg Arg Ala Val Asn His Ile Arg Ser Val Trp

2555 2560 2565

Glu Asn Leu Leu Glu Asp Gln His Thr Pro Ile Asp Thr Thr Ile

2570 2575 2580

Met Ala Lys Asn Glu Val Phe Cys Ile Asp Pro Thr Lys Gly Gly

2585 2590 2595

Lys Lys Pro Ala Arg Leu Ile Val Tyr Pro Asp Leu Gly Val Arg

2600 2605 2610

Val Cys Glu Lys Met Ala Leu Tyr Asp Ile Ala Gln Lys Leu Pro

2615 2620 2625

Lys Ala Ile Met Gly Pro Ser Tyr Gly Phe Gln Tyr Ser Pro Ala

2630 2635 2640

Glu Arg Val Asp Phe Leu Leu Lys Ala Trp Gly Ser Lys Lys Asp

2645 2650 2655

Pro Met Gly Phe Ser Tyr Asp Thr Arg Cys Phe Asp Ser Thr Val

2660 2665 2670

Thr Glu Arg Asp Ile Arg Thr Glu Glu Ser Ile Tyr Gln Ala Cys

2675 2680 2685

Ser Leu Pro Gln Glu Ala Arg Thr Val Ile His Ser Leu Thr Glu

2690 2695 2700

Arg Leu Tyr Val Gly Gly Pro Met Thr Asn Ser Lys Gly Gln Ser

2705 2710 2715

Cys Gly Tyr Arg Arg Cys Arg Ala Ser Gly Val Phe Thr Thr Ser

2720 2725 2730

Met Gly Asn Thr Met Thr Cys Tyr Ile Lys Ala Leu Ala Ala Cys

2735 2740 2745

Lys Ala Ala Gly Ile Val Asp Pro Val Met Leu Val Cys Gly Asp

2750 2755 2760

Asp Leu Val Val Ile Ser Glu Ser Gln Gly Asn Glu Glu Asp Glu

2765 2770 2775

Arg Asn Leu Arg Ala Phe Thr Glu Ala Met Thr Arg Tyr Ser Ala
2780 2785 2790
Pro Pro Gly Asp Leu Pro Arg Pro Glu Tyr Asp Leu Glu Leu Ile
2795 2800 2805
Thr Ser Cys Ser Ser Asn Val Ser Val Ala Leu Asp Ser Arg Gly
2810 2815 2820
Arg Arg Arg Tyr Phe Leu Thr Arg Asp Pro Thr Thr Pro Ile Thr
2825 2830 2835
Arg Ala Ala Trp Glu Thr Val Arg His Ser Pro Val Asn Ser Trp
2840 2845 2850
Leu Gly Asn Ile Ile Gln Tyr Ala Pro Thr Ile Trp Val Arg Met
2855 2860 2865
Val Ile Met Thr His Phe Phe Ser Ile Leu Leu Ala Gln Asp Thr
2870 2875 2880
Leu Asn Gln Asn Leu Asn Phe Glu Met Tyr Gly Ala Val Tyr Ser
2885 2890 2895
Val Asn Pro Leu Asp Leu Pro Ala Ile Ile Glu Arg Leu His Gly
2900 2905 2910
Leu Glu Ala Phe Ser Leu His Thr Tyr Ser Pro His Glu Leu Ser
2915 2920 2925
Arg Val Ala Ala Thr Leu Arg Lys Leu Gly Ala Pro Pro Leu Arg
2930 2935 2940
Ala Trp Lys Ser Arg Ala Arg Ala Val Arg Ala Ser Leu Ile Ala
2945 2950 2955
Gln Gly Ala Arg Ala Ala Ile Cys Gly Arg Tyr Leu Phe Asn Trp
2960 2965 2970
Ala Val Lys Thr Lys Leu Lys Leu Thr Pro Leu Pro Glu Ala Ser
2975 2980 2985
Arg Leu Asp Leu Ser Gly Trp Phe Thr Val Gly Ala Gly Gly Gly

2990 2995 3000

Asp Ile Tyr His Ser Val Ser His Ala Arg Pro Arg Leu Leu Leu

3005 3010 3015

Leu Cys Leu Leu Leu Leu Ser Val Gly Val Gly Ile Phe Leu Leu

3020 3025 3030

Pro Ala Arg

3033

Sequence ID No. 9
Sequence Length: 3,033
Sequence Type: amino acid
Topology: linear
Molecule Type: protein

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Met Ser Thr Asn Pro Lys Pro Gln Arg Lys Thr Lys Arg Asn Thr
 5                  10                  15
Asn Arg Arg Pro Gln Asp Val Lys Phe Pro Gly Gly Gly Gln Ile
 20                  25                  30
Val Gly Gly Val Tyr Leu Leu Pro Arg Arg Gly Pro Arg Leu Gly
 35                  40                  45
Val Arg Ala Thr Arg Lys Thr Ser Glu Arg Ser Gln Pro Arg Gly
 50                  55                  60
Arg Arg Gln Pro Ile Pro Lys Asp Arg Arg Ser Thr Gly Lys Ser
 65                  70                  75
Trp Gly Lys Pro Gly Tyr Pro Trp Pro Leu Tyr Gly Asn Glu Gly
 80                  85                  90
Cys Gly Trp Ala Gly Trp Leu Leu Ser Pro Arg Gly Ser Arg Pro
 95                 100                 105
Thr Trp Gly Pro Thr Asp Pro Arg His Arg Ser Arg Asn Leu Gly
110                 115                 120
Arg Val Ile Asp Thr Ile Thr Cys Gly Phe Ala Asp Leu Met Gly
125                 130                 135
Tyr Ile Pro Val Val Gly Ala Pro Val Gly Gly Val Ala Arg Ala
140                 145                 150
Leu Ala His Gly Val Arg Val Leu Glu Asp Gly Ile Asn Tyr Ala
155                 160                 165
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Thr Gly Asn Leu Pro Gly Cys Ser Phe Ser Ile Phe Leu Leu Ala
170 175 180
Leu Leu Ser Cys Val Thr Met Pro Val Ser Ala Val Glu Val Arg
185 190 195
Asn Ile Ser Ser Ser Tyr Tyr Ala Thr Asn Asp Cys Ser Asn Asn
200 205 210
Ser Ile Thr Trp Gln Leu Thr Asp Ala Val Leu His Leu Pro Gly
215 220 225
Cys Val Pro Cys Glu Asn Asp Asn Gly Thr Leu Arg Cys Trp Ile
230 235 240
Gln Val Thr Pro Asp Val Ala Val Lys His Arg Gly Ala Leu Thr
245 250 255
Arg Ser Leu Arg Thr His Val Asp Met Ile Val Met Ala Ala Thr
260 265 270
Ala Cys Ser Ala Leu Tyr Val Gly Asp Val Cys Gly Ala Val Met
275 280 285
Ile Leu Ser Gln Ala Phe Met Val Ser Pro Gln Arg His Asn Phe
290 295 300
Thr Gln Glu Cys Asn Cys Ser Ile Tyr Gln Gly His Ile Thr Gly
305 310 315
His Arg Met Ala Trp Asp Met Met Leu Asn Trp Ser Pro Thr Leu
320 325 330
Ala Met Ile Leu Ala Tyr Ala Ala Arg Val Pro Glu Leu Val Leu
335 340 345
Glu Ile Ile Phe Gly Gly His Trp Gly Val Ala Phe Gly Leu Gly
350 355 360
Tyr Phe Ser Met Gln Gly Ala Trp Ala Lys Val Val Ala Ile Leu
365 370 375
Leu Leu Val Ala Gly Val Asp Ala Ser Thr Tyr Ser Thr Gly Gln

```
                380                 385                 390
Gln Ala Gly Arg Ala Ala Tyr Gly Ile Ser Ser Leu Phe Asn Thr
                395                 400                 405
Gly Ala Lys Gln Asn Leu His Leu Ile Asn Thr Asn Gly Ser Trp
                410                 415                 420
His Ile Asn Arg Thr Ala Leu Asn Cys Asn Asp Ser Leu Glu Thr
                425                 430                 435
Gly Phe Ile Ala Ser Leu Val Tyr Tyr Arg Arg Phe Asn Ser Ser
                440                 445                 450
Gly Cys Pro Glu Arg Leu Ser Ser Cys Arg Gly Leu Asp Asp Phe
                455                 460                 465
Arg Ile Gly Trp Gly Thr Leu Glu Tyr Glu Thr Asn Val Thr Asn
                470                 475                 480
Asp Glu Asp Met Arg Pro Tyr Cys Trp His Tyr Pro Pro Arg Pro
                485                 490                 495
Cys Gly Ile Val Pro Ala Arg Thr Val Cys Gly Pro Val Tyr Cys
                500                 505                 510
Phe Thr Pro Ser Pro Val Val Val Gly Thr Thr Asp Lys Gln Gly
                515                 520                 525
Val Pro Thr Tyr Thr Trp Gly Glu Asn Glu Thr Asp Val Phe Leu
                530                 535                 540
Leu Asn Ser Thr Arg Pro Pro Arg Gly Ala Trp Phe Gly Cys Thr
                545                 550                 555
Trp Met Asn Gly Thr Gly Phe Thr Lys Thr Cys Gly Ala Pro Pro
                560                 565                 570
Cys Arg Ile Arg Lys Asp Tyr Asn Ser Thr Ile Asp Leu Leu Cys
                575                 580                 585
Pro Thr Asp Cys Phe Arg Lys His Pro Asp Ala Thr Tyr Leu Lys
                590                 595                 600
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Cys Gly Ala Gly Pro Trp Leu Thr Pro Arg Cys Leu Val Asp Tyr
605 610 615
Pro Tyr Arg Leu Trp His Tyr Pro Cys Thr Val Asn Phe Thr Ile
620 625 630
Phe Lys Ala Arg Met Tyr Val Gly Gly Val Glu His Arg Phe Ser
635 640 645
Ala Ala Cys Asn Phe Thr Arg Gly Asp Arg Cys Arg Leu Glu Asp
650 655 660
Arg Asp Arg Gly Gln Gln Ser Pro Leu Leu His Ser Thr Thr Glu
665 670 675
Trp Ala Val Phe Pro Cys Ser Phe Ser Asp Leu Pro Ala Leu Ser
680 685 690
Thr Gly Leu Leu His Leu His Gln Asn Ile Val Asp Val Gln Tyr
695 700 705
Leu Tyr Gly Leu Ser Pro Ala Leu Thr Arg Tyr Ile Val Lys Trp
710 715 720
Glu Trp Val Ile Leu Leu Phe Leu Leu Leu Ala Asp Ala Arg Val
725 730 735
Cys Ala Cys Leu Trp Met Leu Asn Ile Leu Gly Gln Ala Glu Ala
740 745 750
Ala Leu Glu Lys Leu Ile Ile Leu His Ser Ala Ser Ala Ala Ser
755 760 765
Ala Asn Gly Pro Leu Trp Phe Phe Ile Phe Phe Thr Ala Ala Trp
770 775 780
Tyr Leu Lys Gly Arg Val Val Pro Val Ala Thr Tyr Ser Val Leu
785 790 795
Gly Leu Trp Ser Phe Leu Leu Leu Val Leu Ala Leu Pro Gln Gln
800 805 810
Ala Tyr Ala Leu Asp Ala Ala Glu Gln Gly Glu Leu Gly Leu Ala

815 820 825

Ile Leu Val Ile Ile Ser Ile Phe Thr Leu Thr Pro Ala Tyr Lys

830 835 840

Ile Leu Leu Ser Arg Ser Val Trp Trp Leu Ser Tyr Met Leu Val

845 850 855

Leu Ala Glu Ala Gln Ile Gln Gln Trp Val Pro Pro Leu Glu Val

860 865 870

Arg Gly Gly Arg Asp Gly Ile Ile Trp Val Ala Val Ile Leu His

875 880 885

Pro Arg Leu Val Phe Glu Val Thr Lys Trp Leu Leu Ala Ile Leu

890 895 900

Gly Pro Ala Tyr Leu Leu Arg Ala Ser Leu Leu Arg Ile Pro Tyr

905 910 915

Phe Val Arg Ala His Ala Leu Leu Arg Val Cys Thr Leu Val Lys

920 925 930

His Leu Ala Gly Ala Arg Tyr Ile Gln Met Leu Leu Ile Thr Ile

935 940 945

Gly Arg Trp Thr Gly Thr Tyr Ile Tyr Asp His Leu Ser Pro Leu

950 955 960

Ser Thr Trp Ala Ala Gln Gly Leu Arg Asp Leu Ala Ile Ala Val

965 970 975

Glu Pro Val Val Phe Ser Pro Met Glu Lys Lys Val Ile Val Trp

980 985 990

Gly Ala Glu Thr Val Ala Cys Gly Asp Ile Leu His Gly Leu Pro

995 1000 1005

Val Ser Ala Arg Leu Gly Arg Glu Val Leu Leu Gly Pro Ala Asp

1010 1015 1020

Gly Tyr Thr Ser Lys Gly Trp Asn Leu Leu Ala Pro Ile Thr Ala

1025 1030 1035

Tyr Thr Gln Gln Thr Arg Gly Leu Leu Gly Ala Ile Val Val Ser
1040 1045 1050
Leu Thr Gly Arg Asp Lys Asn Glu Gln Ala Gly Gln Val Gln Val
1055 1060 1065
Leu Ser Ser Val Thr Gln Thr Phe Leu Gly Thr Ser Ile Ser Gly
1070 1075 1080
Val Leu Trp Thr Val Tyr His Gly Ala Gly Asn Lys Thr Leu Ala
1085 1090 1095
Gly Pro Lys Gly Pro Val Thr Gln Met Tyr Thr Ser Ala Glu Gly
1100 1105 1110
Asp Leu Val Gly Trp Pro Ser Pro Pro Gly Thr Lys Ser Leu Asp
1115 1120 1125
Pro Cys Thr Cys Gly Ala Val Asp Leu Tyr Leu Val Thr Arg Asn
1130 1135 1140
Ala Asp Val Ile Pro Val Arg Arg Lys Asp Asp Arg Arg Gly Ala
1145 1150 1155
Leu Leu Ser Pro Arg Pro Leu Ser Thr Leu Lys Gly Ser Ser Gly
1160 1165 1170
Gly Pro Val Leu Cys Ser Arg Gly His Ala Val Gly Leu Phe Arg
1175 1180 1185
Ala Ala Val Cys Ala Arg Gly Val Ala Lys Ser Ile Asp Phe Ile
1190 1195 1200
Pro Val Glu Ser Leu Asp Ile Ala Thr Arg Thr Pro Ser Phe Ser
1205 1210 1215
Asp Asn Ser Ala Pro Pro Ala Val Pro Gln Ser Tyr Gln Val Gly
1220 1225 1230
Tyr Leu His Ala Pro Thr Gly Ser Gly Lys Ser Thr Lys Val Pro
1235 1240 1245
Ala Ala Tyr Ala Ser Gln Gly Tyr Lys Val Leu Val Leu Asn Pro

1250 1255 1260

Ser Val Ala Ala Thr Leu Gly Phe Gly Ala Tyr Met Ser Lys Ala

1265 1270 1275

His Gly Ile Asn Pro Asn Ile Arg Thr Gly Val Arg Thr Val Thr

1280 1285 1290

Thr Gly Asp Ser Ile Thr Tyr Ser Thr Tyr Gly Lys Phe Ile Ala

1295 1300 1305

Asp Gly Gly Cys Ala Ala Gly Ala Tyr Asp Ile Ile Ile Cys Asp

1310 1315 1320

Glu Cys His Ser Val Asp Ala Thr Thr Ile Leu Gly Ile Gly Thr

1325 1330 1335

Val Leu Asp Gln Ala Glu Thr Ala Gly Val Arg Leu Val Val Leu

1340 1345 1350

Ala Thr Ala Thr Pro Pro Gly Thr Val Thr Thr Pro His Ser Asn

1355 1360 1365

Ile Glu Glu Val Ala Leu Gly His Glu Gly Glu Ile Pro Phe Tyr

1370 1375 1380

Gly Lys Ala Ile Pro Leu Ala Phe Ile Lys Gly Gly Arg His Leu

1385 1390 1395

Ile Phe Cys His Ser Lys Lys Lys Cys Asp Glu Leu Ala Ala Ala

1400 1405 1410

Leu Arg Gly Thr Gly Val Asn Ala Val Ala Tyr Tyr Arg Gly Leu

1415 1420 1425

Asp Val Ser Val Ile Pro Thr Gln Gly Asp Val Val Val Val Ala

1430 1435 1440

Thr Asp Ala Leu Met Thr Gly Tyr Thr Gly Asp Phe Asp Ser Val

1445 1450 1455

Ile Asp Cys Asn Val Ala Val Ser Gln Ile Val Asp Phe Ser Leu

1460 1465 1470

Asp Pro Thr Phe Thr Ile Thr Thr Gln Thr Val Pro Gln Asp Ala
1475 1480 1485
Val Ser Arg Ser Gln Arg Arg Gly Arg Thr Gly Arg Gly Arg Leu
1490 1495 1500
Gly Ile Tyr Arg Tyr Val Ser Ser Gly Glu Gly Pro Ser Gly Met
1505 1510 1515
Phe Asp Ser Val Val Pro Cys Glu Cys Tyr Asp Ala Gly Ala Ala
1520 1525 1530
Trp Tyr Glu Leu Thr Pro Ala Glu Thr Thr Val Arg Leu Arg Ala
1535 1540 1545
Tyr Phe Asn Thr Pro Gly Leu Pro Val Cys Gln Asp His Leu Glu
1550 1555 1560
Phe Trp Glu Ala Val Phe Thr Gly Leu Thr His Ile Asn Ala His
1565 1570 1575
Phe Leu Ser Gln Thr Lys Gln Gly Gly Glu Asn Phe Ala Tyr Leu
1580 1585 1590
Thr Ala Tyr Gln Ala Thr Val Cys Ala Arg Ala Lys Ala Pro Pro
1595 1600 1605
Pro Ser Trp Asp Val Met Trp Lys Cys Leu Thr Arg Leu Lys Pro
1610 1615 1620
Thr Leu Thr Gly Pro Thr Pro Leu Leu Tyr Arg Leu Gly Ala Val
1625 1630 1635
Thr Asn Glu Val Thr Leu Thr His Pro Val Thr Lys Tyr Ile Ala
1640 1645 1650
Thr Cys Met Gln Ala Asp Leu Glu Ile Met Thr Ser Ser Trp Val
1655 1660 1665
Leu Ala Gly Gly Val Leu Ala Ala Val Ala Ala Tyr Cys Leu Ala
1670 1675 1680
Thr Gly Cys Ile Ser Ile Ile Gly Arg Leu His Leu Asn Asp Arg

1685 1690 1695

Val Val Val Thr Pro Asp Lys Glu Ile Leu Tyr Glu Ala Phe Asp

1700 1705 1710

Glu Met Glu Glu Cys Ala Ser Lys Ala Ala Leu Ile Glu Glu Gly

1715 1720 1725

Gln Arg Met Ala Glu Met Leu Lys Ser Lys Ile Gln Gly Leu Leu

1730 1735 1740

Gln Gln Ala Thr Arg Gln Ala Gln Gly Met Gln Pro Ala Ile Gln

1745 1750 1755

Ser Ser Trp Pro Lys Leu Glu Gln Phe Trp Ala Lys His Met Trp

1760 1765 1770

Asn Phe Ile Ser Gly Ile Gln Tyr Leu Ala Gly Leu Ser Thr Leu

1775 1780 1785

Pro Gly Asn Pro Ala Val Ala Ser Met Met Ala Phe Ser Ala Ala

1790 1795 1800

Leu Thr Ser Pro Leu Pro Thr Ser Thr Thr Ile Leu Leu Asn Ile

1805 1810 1815

Met Gly Gly Trp Leu Ala Ser Gln Ile Ala Pro Pro Ala Gly Ala

1820 1825 1830

Thr Gly Phe Val Val Ser Gly Leu Val Gly Ala Ala Val Gly Ser

1835 1840 1845

Ile Gly Leu Gly Lys Ile Leu Val Asp Val Leu Ala Gly Tyr Gly

1850 1855 1860

Ala Gly Ile Ser Gly Ala Leu Val Ala Phe Lys Ile Met Ser Gly

1865 1870 1875

Glu Lys Pro Thr Val Glu Asp Val Val Asn Leu Leu Pro Ala Ile

1880 1885 1890

Leu Ser Pro Gly Ala Leu Val Val Gly Val Ile Cys Ala Ala Ile

1895 1900 1905

Leu Arg Arg His Val Gly Gln Gly Glu Gly Ala Val Gln Trp Met
1910 1915 1920
Asn Arg Leu Ile Ala Phe Ala Ser Arg Gly Asn His Val Ala Pro
1925 1930 1935
Thr His Tyr Val Val Glu Ser Asp Ala Ser Gln Arg Val Thr Gln
1940 1945 1950
Val Leu Ser Ser Leu Thr Ile Thr Ser Leu Leu Arg Arg Leu His
1955 1960 1965
Ala Trp Ile Thr Glu Asp Cys Pro Ile Pro Cys Ser Gly Ser Trp
1970 1975 1980
Leu Gln Asp Ile Trp Asp Trp Val Cys Ser Ile Leu Thr Asp Phe
1985 1990 1995
Lys Asn Trp Leu Ser Ser Lys Leu Leu Pro Lys Met Pro Gly Ile
2000 2005 2010
Pro Phe Ile Ser Cys Gln Lys Gly Tyr Lys Gly Val Trp Ala Gly
2015 2020 2025
Thr Gly Val Met Thr Thr Arg Tyr Pro Cys Gly Ala Asn Ile Ser
2030 2035 2040
Gly His Val Arg Met Gly Thr Met Lys Ile Thr Gly Pro Lys Thr
2045 2050 2055
Cys Leu Asn Leu Trp Gln Gly Thr Phe Pro Ile Asn Cys Tyr Thr
2060 2065 2070
Glu Gly Pro Cys Val Pro Lys Pro Pro Pro Asn Tyr Lys Thr Ala
2075 2080 2085
Ile Trp Arg Val Ala Ala Ser Glu Tyr Val Glu Val Thr Gln His
2090 2095 2100
Gly Ser Phe Ser Tyr Val Thr Gly Leu Thr Ser Asp Asn Leu Lys
2105 2110 2115
Val Pro Cys Gln Val Pro Ala Pro Glu Phe Phe Ser Trp Val Asp

2120 2125 2130

Gly Val Gln Ile His Arg Phe Ala Pro Val Pro Gly Pro Phe Phe

2135 2140 2145

Arg Asp Glu Val Thr Phe Thr Val Gly Leu Asn Ser Phe Val Val

2150 2155 2160

Gly Ser Gln Leu Pro Cys Asp Pro Glu Pro Asp Thr Glu Val Leu

2165 2170 2175

Ala Ser Met Leu Thr Asp Pro Ser His Ile Thr Ala Glu Ala Ala

2180 2185 2190

Ala Arg Arg Leu Ala Arg Gly Ser Pro Pro Ser Gln Ala Ser Ser

2195 2200 2205

Ser Ala Ser Gln Leu Ser Ala Pro Ser Leu Lys Ala Thr Cys Thr

2210 2215 2220

Thr His Lys Thr Ala Tyr Asp Cys Asp Met Val Asp Ala Asn Leu

2225 2230 2235

Phe Met Gly Gly Asp Val Thr Arg Ile Glu Ser Asp Ser Lys Val

2240 2245 2250

Ile Val Leu Asp Ser Leu Asp Ser Met Thr Glu Val Glu Asp Asp

2255 2260 2265

Arg Glu Pro Ser Val Pro Ser Glu Tyr Leu Ile Lys Arg Arg Lys

2270 2275 2280

Phe Pro Pro Ala Leu Pro Pro Trp Ala Arg Pro Asp Tyr Asn Pro

2285 2290 2295

Val Leu Ile Glu Thr Trp Lys Arg Pro Gly Tyr Glu Pro Pro Thr

2300 2305 2310

Val Leu Gly Cys Ala Leu Pro Pro Thr Leu Gln Thr Pro Val Pro

2315 2320 2325

Pro Pro Arg Arg Arg Arg Ala Lys Ile Leu Thr Gln Asp Asp Val

2330 2335 2340

Glu Gly Ile Leu Arg Glu Met Ala Asp Lys Val Leu Ser Pro Leu
2345 2350 2355
Gln Asp Asn Asn Asp Ser Gly His Ser Thr Gly Ala Asp Thr Gly
2360 2365 2370
Gly Asp Ile Val Gln Gln Pro Ser Asp Glu Thr Ala Ala Ser Glu
2375 2380 2385
Ala Gly Ser Leu Ser Ser Met Pro Pro Leu Glu Gly Glu Pro Gly
2390 2395 2400
Asp Pro Asp Leu Glu Phe Glu Pro Val Gly Ser Ala Pro Pro Ser
2405 2410 2415
Glu Gly Glu Cys Glu Val Ile Asp Ser Asp Ser Lys Ser Trp Ser
2420 2425 2430
Thr Val Ser Asp Gln Glu Asp Ser Val Ile Cys Cys Ser Met Ser
2435 2440 2445
Tyr Ser Trp Thr Gly Ala Leu Ile Thr Pro Cys Gly Pro Glu Glu
2450 2455 2460
Glu Lys Leu Pro Ile Asn Pro Leu Ser Asn Ser Leu Met Arg Phe
2465 2470 2475
His Asn Lys Val Tyr Ser Thr Thr Ser Arg Ser Ala Ser Leu Arg
2480 2485 2490
Ala Lys Lys Val Thr Phe Asp Arg Val Gln Val Leu Asp Ala His
2495 2500 2505
Tyr Asp Ser Val Leu Gln Asp Val Lys Arg Ala Ala Ser Lys Val
2510 2515 2520
Gly Ala Arg Leu Leu Thr Val Glu Glu Ala Cys Ala Leu Thr Pro
2525 2530 2535
Pro His Ser Ala Lys Ser Arg Tyr Gly Phe Gly Ala Lys Glu Val
2540 2545 2550
Arg Ser Leu Ser Arg Arg Ala Val Asn His Ile Arg Ser Val Trp

2555 2560 2565

Glu Asn Leu Leu Glu Asp Gln Arg Thr Pro Ile Asp Thr Thr Ile

2570 2575 2580

Met Ala Lys Asn Glu Val Phe Cys Ile Asp Pro Thr Lys Gly Gly

2585 2590 2595

Lys Lys Pro Ala Arg Leu Ile Val Tyr Pro Asp Leu Gly Val Arg

2600 2605 2610

Val Cys Glu Lys Met Ala Leu Tyr Asp Ile Thr Gln Lys Leu Pro

2615 2620 2625

Lys Ala Ile Met Gly Pro Ser Tyr Gly Phe Gln Tyr Ser Pro Ala

2630 2635 2640

Glu Arg Val Asp Phe Leu Leu Lys Ala Trp Gly Ser Lys Lys Asp

2645 2650 2655

Pro Met Gly Phe Ser Tyr Asp Thr Arg Cys Phe Asp Ser Thr Val

2660 2665 2670

Thr Glu Arg Asp Ile Arg Thr Glu Glu Ser Ile Tyr Gln Ala Cys

2675 2680 2685

Ser Leu Pro Gln Glu Ala Arg Thr Val Ile His Ser Leu Thr Glu

2690 2695 2700

Arg Leu Tyr Val Gly Gly Pro Met Thr Asn Ser Lys Gly Gln Ser

2705 2710 2715

Cys Gly Tyr Arg Arg Cys Arg Ala Ser Gly Val Phe Thr Thr Ser

2720 2725 2730

Met Gly Asn Thr Met Thr Cys Tyr Ile Lys Ala Leu Ala Ala Cys

2735 2740 2745

Lys Ala Ala Gly Ile Val Asp Pro Val Met Leu Val Cys Gly Asp

2750 2755 2760

Asp Leu Val Val Ile Ser Glu Ser Gln Gly Asn Glu Glu Asp Glu

2765 2770 2775

Arg Asn Leu Arg Ala Phe Thr Glu Ala Met Thr Arg Tyr Ser Ala
2780 2785 2790
Pro Pro Gly Asp Leu Pro Arg Pro Glu Tyr Asp Leu Glu Leu Ile
2795 2800 2805
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Arg Ala Ala Trp Glu Thr Val Arg His Ser Pro Val Asn Ser Trp
2840 2845 2850
Leu Gly Asn Ile Ile Gln Tyr Ala Pro Thr Ile Trp Val Arg Met
2855 2860 2865
Val Ile Met Thr His Phe Phe Ser Ile Leu Leu Ala Gln Asp Thr
2870 2875 2880
Leu Asn Gln Asn Leu Asn Phe Glu Met Tyr Gly Ala Val Tyr Ser
2885 2890 2895
Val Asn Pro Leu Asp Leu Pro Ala Ile Ile Glu Arg Leu His Gly
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Arg Val Ala Ala Thr Leu Arg Lys Leu Gly Ala Pro Pro Leu Arg
2930 2935 2940
Ala Trp Lys Ser Arg Ala Arg Ala Val Arg Ala Ser Leu Ile Ala
2945 2950 2955
Gln Gly Ala Arg Ala Ala Ile Cys Gly Arg Tyr Leu Phe Asn Trp
2960 2965 2970
Ala Val Lys Thr Lys Leu Lys Leu Thr Pro Leu Pro Glu Ala Ser
2975 2980 2985
Arg Leu Asp Leu Ser Gly Trp Phe Thr Val Gly Ala Gly Gly Gly